National Institute
of
Allergy and Infectious
Diseases

Annual Report of Intramural Activities

October 1, 1989-September 30, 1990

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

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of
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Public Health Service
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For Administrative Use

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NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1990 ANNUAL REPORT OF INTRAMURAL ACTIVITIES

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1990 ANNUAL REPORT PROJECT NUMBER LISTING OF ACTIVE PROJECTS

Z01-AI
00013-27 LIP
00020-15 ODIR
00027-23 LMM
00030-22 LI
00035-15 LI
00036-25 LI
00043-25 LCI
00045-22 LCI
00047-21 LCI
00048-20 LCI
00057-17 LCI
00058-16 LCI
00072-19 LPVD
00074-18 LPVD
00085-13 LPVD
00086-13 LPVD
00094-31 LPD
00097-32 LPD
00098-34 LPD
00099-20 LPD
00102-16 LPD
00108-19 LPD
00123-24 LVD
00126-17 LVD
00134-28 LI
00135-16 LIP
00143-21 LIG
00144-26 LIG
00145-23 LIG
00154-15 LCI
00155-15 LCI
00161-13 LPD
00162-14 LPD
00166-13 LIG
00168-13 LIG
00169-13 BRB
00170-13 LIG
00171-13 LIG
00172-12 BRB
00180-12 LIG
00182-12 ODIR
00183-12 ODIR
00190-12 LMM
00193-11 LMSF
00197-11 LPD
00208-10 LPD
00210-10 LIR
00213-10 LIR
00216-10 LICP

Z01-AI
00218-09 LMM
00224-09 LI
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00244-09 LPD
00246-08 LPD
00248-09 LPD
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00342-09 LID
00343-09 LID
00345-09 LID

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1990 ANNUAL REPORT PROJECT NUMBER LISTING OF ACTIVE PROJECTS

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00349-08 LI
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00354-08 LCI
00356-08 LCI
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00361-08 LIR
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00370-08 LID
00372-08 LID
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00390-07 LIR
00394-07 LI
00397-07 LCI
00403-07 LI
00415-06 LMM
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00418-07 LPVD
00425-06 LI
00427-06 LI
00429-06 LCI
00430-06 LCI
00431-06 LIR
00432-06 LCI
00437-06 LMM
00438-06 LMM
00439-06 LPD
00441-06 LICP
00445-06 LVD
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00487-04 LPD
00488-04 LVP

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00551-02 LPVD
00552-02 LVP
00553-02 LVP
00554-02 LVP
00555-02 LID

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1990 ANNUAL REPORT PROJECT NUMBER LISTING OF ACTIVE PROJECTS

Z01-AI
00556-02 LID
00557-02 LID
00558-02 LID
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00564-02 LVD
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00569-01 LID
00570-01 LID
00571-01 LID
00572-01 LID
00573-01 LID
00574-01 LID
00575-01 LID
00576-01 LID
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00578-01 LIP
00579-01 LPD
00580-01 LPVD
00581-01 LCMI
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00583-01 LID
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00585-01 LIR
00586-01 LIR
00587-01 LIR
00588-01 LMM
00589-01 LCI
00590-01 LCI
00591-01 LCI
00592-01 LCI
00593-01 LCI
00594-01 ODIR

OFFICE OF THE DIRECTOR, DIVISION OF INTRAMURAL RESEARCH, NIAID
1990 Annual Report
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SUMMARY OF PROGRAM
Laboratory and Clinical Research NIAID
October 1, 1989 - September 30, 1990

The Division of Intramural Research (DIR), NIAID consists of 14 laboratories, a Biological Resources Branch, Administrative Management Branch and Animal Care Branch. Four laboratories are located in Hamilton, Montana at the Rocky Mountain Laboratories. The remaining facilities are concentrated at the NIH Bethesda Campus with auxiliary facilities at Frederick (Ft. Detrick) and Rockville, MD. The Office of the Director, DIR, (also called the Office of the Scientific Director, NIAID) is responsible for the management of the Division of Intramural Research as well as advising the Director, NIAID, on scientific issues. In FY'90 the DIR, NIAID, had an allocation of $107,312,000 with 39.47% for AIDS research and 60.53% for other than AIDS research. The allocations of the budget were as follows; 29.90% for salaries, 44.25% for laboratory supplies, contracts (supply and R&D) and administrative support, 7.24% for the management fund and 18.6% for inpatient and outpatient care costs. In FY'90 there were over 850 persons working within DIR, NIAID, with 92 tenured scientists, 568 nontenured scientists (including guest workers and special volunteers) and 198 nonscientists.

Numerous outstanding accomplishments were made by DIR, NIAID scientists and are summarized in this annual report. Some of the many highlights were as follows:

RESEARCH OTHER THAN AIDS

o Inflammatory Bowel Disease (IBD) is a severe disease of the intestines of unknown cause. IBD affects 1-2 million patients and is treated with drugs that have multiple side effects. It has been discovered that this disease may be mediated by specialized T cells that normally protect patients against infections.

o A new recombinant vaccine against Herpes Simplex type 2 (HSV-2) has successfully completed its initial trials in human volunteers and will be tested in patients infected with genital herpes caused by HSV-2.

o Interferon gamma was shown to be efficacious in reducing the number and severity of infections in chronic granulomatous disease (CGD) of childhood. Based on these studies, for the first time the Food and Drug Administration recommended interferon gamma to treat an immune deficiency.

o The gene which is lacking in autosomal recessive Chronic Granulomatous Disease (ARCGD) patients has been successfully transplanted into human and mouse cells. The implication of these experiments is that treatment of AR-CGD by gene therapy is possible.

o Common structures in the Major Outer Membrane Protein (MOMP) have been identified that will be examined for their utility in developing a single vaccine which is effective against all forms of Chlamydial infections.

- The Major Histocompatibility Complex II, types DR and DP, had been discovered to have different protein structures. This differentiation has been found to be significant because the bacterial toxin that causes toxic shock syndrome binds only to the DR type.

- Transforming Growth Factor-β was discovered to have an important role in causing malfunction of the immune system in patients with T cell leukemia.

- Using a specialized molecular biology technique called polymerase chain reaction, it will be possible to identify, on site, regions of the world which have chloroquine resistant malaria.

- Resting B cells in the mouse model induce a state of tolerance to antigens.

- Interleukin 4 is an essential cell secreted chemical that regulates the release of antibodies associated with allergic reactions (IgE). Using different tests, one of the mechanisms for inducing the expression of IL-4 in cells has been isolated.

- Candidate vaccines for dengue have been developed.

- Liver cancer associated with hepatitis is the leading cause of cancer related death in most developing countries. The woodchuck model has been recently developed enough to be used in helping scientists isolate the hepatitis virus gene that causes liver cancer.

- Polymerase chain reaction, a molecular biology technique used to amplify small bits of molecular material, has been used to help determine the exact gene sequence of the human chromosomes. The largest problem has been finding genetic "signposts" to help researchers map gene positions on the chromosome structure. The detection of the *alu* sequences using PCR has been developed to isolate and identify these position markers.

- Group B Streptococcal infections cause severe infections in infants shortly after birth. Early progress in developing therapy to prevent and cure this severe pediatric infection using interleukins has been made.

- *Borrelia burgdorferi,* the agent that causes Lyme disease, is seldom isolated from infected patients, and current serological tests are often inconclusive because of the presence of cross-reacting non-*B. burgdorferi* antibodies. Using molecular biology techniques, a protein named P39 has been isolated and when used in serum tests for Lyme disease, has been shown to be very sensitive in detecting infected individuals.

- Studies of the enzyme systems of the *Bordetella* bacteria have revealed new toxins that may be used in the development of good vaccines against whooping cough.

AIDS RESEARCH

- o CD4 combined with a bacterial toxin are selective killers of HIV infected cells. Preliminary data indicates that this compound may act synergistically with current medications used to treat AIDS.

- o Rabbits infected with HIV-1 have impaired immune response to certain antigens.

- o The brain was shown to be a preferential target of HIV-1 infection in the rabbit.

- o The protein substances that cells secrete to induce immune functions in other cells, cytokines, have been discovered to induce increased replication of HIV in cells chronically infected with retroviruses. This observation has clarified to some degree understanding of why HIV disease progresses in such an insidious manner.

- o A new protein called p75 has some of the same HIV protective properties of gp120 vaccines. It may be a good candidate for vaccine development.

- o Early clinical studies indicate that a candidate AIDS vaccine using a specialized replication system for gp160 induces significant cellular and antibody immune responses to HIV.

- o Alpha interferon decreases the rate of appearance of opportunistic infections in HIV + patients and preserves the level of CD4+ cells for a longer period of time.

- o The NF-*k*B protein produced by the HIV molecule has been found to be one of the crucial elements that permits the HIV virus to replicate. Using selective molecular biology techniques, NIAID scientists have proven that 1) NF-*k*B and Sp1 are necessary for replication 2) NF-*k*B alone can allow replication of HIV but at a slower pace 3) replication is not possible with only Sp1. Isolation of the controlling site of HIV replication will allow development of new strategies for HIV treatment.

- o NIAID scientists studying the murine leukemia virus (MuLV) as a model for AIDS have discovered that the virus induces oncogenes in mouse cells. In a second study, the sites flanking the area where HIV inserts its genes into the host genes are commonly found to be a type of oncogene called *ras*. This association may explain why HIV patients have a higher prevalence of cancers.

Equal Employment Opportunity and affirmative Action Programs have been given a high level of attention this year. Our annual Introduction to Biomedical Research Program for Minority Students from across the U.S. and Puerto Rico was successful in providing the students with a brief introduction to biomedical research. The Director, DIR, NIAID and the Special Assistant to the Director, DIR, NIAID have visited and spoken with the Deans and faculty of the minority medical schools to recruit minority scientists for the NIAID research program. Three minority physicians

were recruited to the clinical research program. This year in the annual minority summer program for high school, college and medical students, 64 student worked in the NIAID laboratories; 40.6% of the students in the summer program were minorities.

The NIAID Board of Scientific Counselors under the leadership of Dr. Stanley Falkow reviewed the Laboratory of Molecular Microbiology (Dr. Malcolm Martin, Chief), and the Laboratory of Viral Diseases (Dr. Bernard Moss, Chief), on December 11-13, 1989 and the Laboratory of Clinical Investigation (Dr. Michael Frank, Chief) on May 21-23, 1990. All of these laboratories received excellent reviews. This year Drs. Gerald L. Mandell and Raphael Dolin completed their tenure on the Board and Dr. Michael Frank, NIAID Clinical Director and Chief, Laboratory of Clinical Investigation, will be leaving the NIAID to become Professor and Chairman of the Department of Pediatrics, Duke University, Durham, North Carolina. The services of Drs. Mandell and Dolin on the Board of Scientific Counselors and Dr. Frank have been greatly appreciated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00594-01 ODIR

PERIOD COVERED
February 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Function of Fcγ and Fcε receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jean Pierre Kinet, Section Chief - MAIS, OSD, NIAID

Anna Teresa Brin, Visiting Assoc.
Marie-Helene Jouvin, Visiting Assoc.
Helmut Kuster, Visiting Assoc.
Li Zhang, Visiting Assoc.
Odile Mejan, Visiting Fellow
Rossella Paolini, Visiting Fellow
Fumiyoski Takizawa, Visiting Fellow
Bernie Effertz, IRTA

COOPERATING UNITS *(if any)*
Arthritis Branch, NIAMS
Laboratory of Clinical Investigation, NIAID

LAB/BRANCH
Division of Intramural Research

SECTION
Molecular Allergy and Immunology Section

INSTITUTE AND LOCATION
NIAID, Twinbrook II, NIH Rockville, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9	9	2 summer students

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The receptor with high affinity for IgE (FcεRI) is a key molecule in triggering the allergic reaction. Therefore understanding its structure and its function is of considerable medical interest and may lead to new therapeutic approaches of allergic diseases. During the past year we have engaged in several types of studies: on the gene structure of FcεRI, studies on the expression of the receptor by gene transfer, studies on the expression of the receptor during cell differentiation, studies on the receptor and IgE binding sites and studies on the structural relationship between FcεRI and Fcγ receptors. These studies have yielded the following results: 1. With the exception of human β, cDNA clones for α, β and γ of FcεRI have now been isolated from three different species: rat, mouse and human. The genomic structure of the human γ gene has been defined and the chromosomal localization of the subunits has been completed in mice and in human. 2. By using the techniques of gene transfer, non receptor bearing cells have been induced to express receptors on their surface. The requirements for cell surface expression are different for the rodent and the human receptor. To understand this difference chimeric (rodent-human) α chains and other mutants of α have been produced. 3. Receptor expression comes at a very early stage of mouse mast cells and human basophils differentiation in IL3-dependent bone marrow cell cultures. 4. To eventually allow production of crystals to study the receptor binding site, we have engineered truncated human α chains which are secreted by COS 7 cells. Production of a stable cell line has also been accomplished. 5. Fc receptors from mouse macrophages and human NK cells are physically associated with γ chains identical to those from FcεRI. These γ chains have been cloned. Their presence is required for the expression of these FcεRs in transfected cells. 6. The γ chains also associate with the ζ and η chains of the T cell receptor. They define a new family of dimers which may be involved in important functions of signal transduction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00020-15-ODIR

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies on the Treatment of Disease with the Interferon System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Hilton B. Levy, Ph.D.	Special Assistant to the Scientific Director	NIAID

COOPERATING UNITS (if any) Dr. A. Salazar and others at Walter Reed Army Hospital; Dr. J. Morales, Presbyterian Hospital, Puerto Rico; Dr. S. Baron, U. of Texas Medical School, Galveston, TX; Virology and Disease Control Divisions, USAMRIID.

LAB/BRANCH
Office of Director

SECTION

INSTITUTE AND LOCATION
NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS: 1.7	PROFESSIONAL: 1.0	OTHER: 0.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Clinical studies using PolyICLC with Dr. Salazar of Walter Reed include three diseases. 1) Preliminary studies with AIDS. There are currently 35 patients who have been entered into the first phase of the work determining the best dose and schedule. Detailed analysis of lymphocyte subsets showed that there are transient rises in a number of subsets after injection of polyICLC. This effect lasts for one or two days, and then the value reverts to pretreatment levels. We have started a number of patients on polyICLC alone, without AZT, 3 times a week, at a low dose. There was one death in this group of six, a patient who had pneumocystis before starting treatment and who died a week after starting treatment. There have been no opportunistic infections in this group, but this schedule has been ongoing only 4 months. 2) The work with multiple sclerosis is developing to the point where a randomized double-blind trial may be considered. Patients receive low doses of the drug 2 or 3 times a week. There are minimal side effects, and the patients either remain stable or improve slightly. The first patient with neurologic disease, who was put on polyICLC nine years ago, made a dramatic improvement. He was treated for seven years. Starting with complete paralysis, after treatment for three months he was able to go home, complete his education, get a job, and get married; however, he experienced recurring weakness about every 5-6 weeks and needed polyICLC treatment. Now, he has been off the drug for 1.5 years and remains well. 3) Five glioma patients are being treated with polyICLC plus CCNU in an adjuvant setting. One of the five died after a year; the others are still alive.

USAMRIID has initiated a formal plan to treat normal volunteers prophylactically with polyICLC to test its efficacy against exotic virus infections of military importance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00182-12 ODIR

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical & Genetic Mechanisms of Obligate Intracellular Parasitism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.C. Williams, Ph.D., Sr. Scientist, NIAID/ODIR, Chief, Intracellular Pathogens Branch, Bacteriology Division, USAMRIID.

T. A. Hoover, Ph.D., USAMRIID.

N. Bhatnagar, Ph.D., National Research Council Fellow

S.-Y. Chen, M.S., Pre-doctoral candidate.

C. R. Bolt, M.S., USAMRIID

COOPERATING UNITS (if any)

M. H. Vodkin, Ph.D., University of Illinois, Urbana, IL.

H. A. Thompson, Ph.D., Visiting Scientist and Associate Professor, Dept. Microbiol and Immunol., West Virginia University Medical Center, Morgantown, WV.

LAB/BRANCH

Office of the Director of Intramural Research Programs, NIAID, Bethesda, MD

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD and Bldg. 550, Ft. Detrick, Frederick, MD 21702-5011

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER.
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project in this reporting period were i) the cloning and DNA sequencing of the gene (pyrB) encoding aspartate transcarbamylase (ATCase) and the analysis of the gene product, ii) the cloning and DNA sequencing of a C. burnetii insertion sequence (IS), iii) the development of a physical map of the C. burnetii chromosome, iv) the analysis of the induction of heat-shock proteins, and v) the analysis of DNA synthesis during acid-activation of C. burnetii. A. The pyrB gene encodes a catalytically active ATCase that is homologous with other ATCases only at the active site and without a regulatory subunit. B. The IS of C. burnetii is similar though not identical to other bacteria and present in 15-17 copies per genome. C. Variant chromosomal banding among strains correlates with degrees of virulence of C. burnetii. D. Heat-shock (or stress) proteins which are metabolic signals of temperature shifts are synthesized and a 62 kDa protein is exported. E. The condition of acid activation triggers the synthesis of both chromosomal and plasmid DNA. Significance. i) C. burnetii ATCase, as predicted by the DNA sequence of pyrB, is not highly regulated at the level of enzyme activity. Aspects of developmental control of ATCase synthesis can now be studied with the pyrB gene probe. ii) The IS of C. burnetii appears to be unique and with an unusually high number of copies per genome. The encoded basic protein has characteristics of a DNA binding protein which may facilitate genetic recombination experimentation. iii) Analysis of the physical maps of C. burnetii revealed a genome size of 1750 Kb pairs and differences among various strains of known virulence. iv) C. burnetii responds to an increase in temperature and acid activation by synthesizing a variety of heat-shock proteins, chromosomal and plasmid DNA. These markers of metabolic activation will be used to study the adaptation of C. burnetii to life in the phagolysosome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00183-12 ODIR

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunologic Properties of Coxiella burnetii (Q Fever) Vaccines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J. C. Williams, Ph.D., Sr. Scientist, NIAID/ODIR, Chief, Intracellular Pathogens Branch, Bacteriology Division, USAMRIID
G. H. Scott, Ph.D., USAMRIID
D. M. Waag, Ph.D., USAMRIID
C. R. Bolt, M.S., USAMRIID
T. A. Hoover, Ph.D., USAMRIID
T. F. McCaul, Ph.D., SR, Fellow National Research Council Fellow
N. Bhatnagar, Ph.D., National Research Council Fellow
G. Sandstrom, Ph.D., National Research Council Fellow

COOPERATING UNITS (if any)

H. A. Thompson, Ph.D., Dept Microbiol & Immunol, WV Univ Med Ctr, Morgantown, WV
J. M. Blondeau, Ph.D., T. J. Marrie, M.D., J. Embil, M.D., Dept Med, Dalhousie University, Halifax, N.S., Canada

LAB/BRANCH

Office of the Director of Intramural Research Programs, NIAID, Bethesda, MD

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD AND Bldg. 550, Ft. Detrick, Frederick, MD 21702-5011

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither
[ ] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project in this reporting period were i) the evaluation of the safety and immunogenicity of the Francisella tularensis live vaccine in human subjects, ii) to study the changes in murine splenic and thymic lymphoid populations after injection with Coxiella burnetii antigens, iii) the evaluation of C. burnetii lipopolysaccharide (LPS) and protein antigens as vaccines against Q fever, and iv) to study the humoral antibody immune response of humans naturally infected with C. burnetii. A. The scarification vaccination of humans with F. tularensis produced mild local reactions at the inoculation site, a significant rise in specific IgA, IgG and IgM antibodies, and a marked increase in lymphocytes responding to specific F. tularensis antigens. B. The injection of mice with phase I C. burnetii whole cells induced significant lymphocyte immunosuppression to mitogens and antigens without a significant change in antibody levels. The whole cell antigen apparently activated resident suppressor T cells rather than induce an increase in the number of Lyt-2 cells. C. Although the LPS was effective as a protective antigen, a major surface protein was more protective than LPS. D. The analysis of a specific humoral immune response by immunoblotting with human Q fever sera revealed that IgM was the first antibody to be produced, then IgG and IgA. The early detection of IgM and IgG to phase I and II whole cells in acute Q fever contrasted sharply with the lack of IgA antibodies against the LPS. Significance. i) The intradermal administration of the F. tularensis live vaccine to humans appears to be safe and it induced significant cell-mediated and humoral immune responses, ii) The suppression of cell-mediated immune responses after the injection of mice with phase I whole cells is unique in that it activates resident suppressor cells rather than altering the balance of suppressors and helpers, iii) Protein antigens of C. burnetii are effective vaccines against Q fever in mice, iv) detection of specific antibodies against several proteins but not of antibodies against the phase I lipopolysaccharide by immunoblotting distinguishes acute from chronic Q fever.

PHS 6040 (Rev. 1/84)

ANIMAL CARE BRANCH

ANNUAL REPORT FY 1990

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ANIMAL CARE BRANCH

1990 Annual Report

Summary

Much of the current research in the understanding and treatment of allergies and infectious diseases requires the use of laboratory animals. The care and treatment of these animals can have a profound affect on the results of experimental procedures. An animal's environmental conditions, its daily care, the presence or absence of disease-causing microorganisms, the amount of pain or distress that results from experimental manipulations all may affect the validity of research data. The NIAID intramural scientists today are very sensitive to these factors and their ethical responsibility for ensuring that animals used in research receive high quality animal care. All NIAID scientists conducting research that involves animals are required to attend the NIH course on "Using Animals in Intramural Research: Guidelines for Investigators." This course offers not only a broad perspective on current animal issues but also provides specific information on actual techniques and procedures.

It is the responsibility of the NIAID Animal Care Branch to provide the daily care to all animals maintained within the NIAID animal facilities, to oversee NIAID's intramural contracts involving animals, and to assist NIAID scientists with animal-related problems. The NIAID maintains five animal facilities at the NIH campus in Bethesda, Maryland; one animal facility in Rockville, Maryland; two animal facilities at the Frederick Cancer Research Development Center in Frederick, Maryland; and four animal facilities at Rocky Mountain Laboratories in Hamilton, Montana. Approximately 58,000 square feet of space (26,000 sq. ft. at the NIH in Bethesda, MD; 2,500 sq. ft. at the FCRDC in Frederick, MD; and 29,000 sq. ft. in Hamilton, MT), excluding pasture land, are devoted to the NIAID Animal Care Program.

The NIAID's Animal Care Branch provides guidance to the Institute's intramural scientists using animals in research projects. This guidance includes assistance in the purchase of animals, the selection and proper administration of anesthetics and analgesics, the diagnosis, treatment and control of infectious agents, and the performance of technical procedures in laboratory animals. The Branch maintains production colonies of over sixty different strains of mice, hamsters, cotton rats, and rabbits for NIAID investigators within DIR or contract animal facilities. Many of these animals are unavailable anywhere else in the world or are available only after long delays. The NIAID Division of Intramural Research is committed to the use of animals only when alternative methods are unavailable, and fully supports existing Federal rules and regulations pertaining to the care and use of animals in biomedical research. The Institute has hired three additional veterinarians to help manage its' animal care and use program and has spent, in FY90, over $3,500,000 in upgrading its' animal facilities and purchasing new animal equipment.

ADMINISTRATIVE MANAGEMENT BRANCH

ANNUAL REPORT FY 1990

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ADMINISTRATIVE MANAGEMENT BRANCH, DIR
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
October 1, 1989 to September 30. 1990

Introduction

The mission of the Division of Intramural Research (DIR) is multifaceted (1) to conduct basic research and applied research on immunologic and allergic diseases, infectious diseases and related clinical disorders; (2) to provide expertise in each of these areas who can advise the NIAID, PHS, and DHHS; and (3) to provide training in both clinical and basic biomedical research for young scientists in the disease areas within the Institute's realm of responsibility. The Division of Intramural Research's Administrative Management Branch (AMB) was established within the Office of the Director, in June 1986. The AMB provides the critical linkage between general administrative issues and scientific program management, development and priorities. The Branch is responsible for planning, directing and coordinating the administrative management functions for the Division of Intramural Research including personnel management, financial management, space management, procurement, and other daily functions necessary to ensure the efficient and effective implementation of research objectives. Each DIR laboratory and branch has its own group of administrative and procurement staff to provide day-to-day management and administrative assistance. Over the years, the Administrative Management Branch has been able to provide the DIR with the highest quality administrative support and advisory services possible. The following is a brief exposition of some of our accomplishments and activities during fiscal year 1990.

Budget and Financial Management

In FY '90 the DIR budget allocation was increased by 15 percent over the FY '89 level, to a total budget of $107,012,000. Forty percent of this budget was allocated for support of AIDS and AIDS-related research, including the HOPE Amendment which earmarked funds for the AIDS Outpatient expansion. The AMB was responsible for the accounting and monitoring of these funds in each specific category.

During FY '90 a new budget tracking system, termed Status of Funds (**SOF**), was developed and implemented. With this system, AMB has developed the capacity for accessing financial data from the Division of Financial Management (DFM) directly on-line, obviating the cumbersome paper printouts previously used. The SOF has extensive flexibility and several types of reports were developed to provide detailed data to the laboratories to assist them in the management of their resources, and to insure the appropriateness of the costs assigned to them. The system, in conjunction with the Administrative Management and Budget Information System (AMBIS), has allowed for better monitoring and understanding of in-house (S&SF) charges, which historically have been classified as "non-trackable." The on-line system is available to all NIAID Administrative Offices to support their respective laboratories and branches. AMBIS is also available to all staff in NIAID labs/branches within their designated areas. This system has streamlined not only the Institute procurement process but also the budget

monitoring process. Both AMBIS and the SOF were developed to work in concert to provide complete support for the DIR staff in the management of the budget, and have been well received by all users.

Renovations and Space Relocations

The AMB is responsible for studying space allocations in DIR, concentrating on efficient flow of work as it relates to space assignments. Other responsibilities relative to space include insuring the adequacy of resources for renovations, coordinating laboratory renovations with the Division of Engineering Services (DES), budgeting of program and/or construction monies for such renovations, and meeting with the appropriate NIH officials to resolve problems. The Branch continued to play a major role in coordinating and monitoring the progress of many space renovations and relocations this fiscal year.

The DES Phase 1 renovation of the Twinbrook II facility will be completed in August of this fiscal year. This renovation provides 15,000 square feet of new state-of-the-art laboratory space for DIR scientists located at this facility. The renovation includes 1,640 square feet of BL-3 laboratory space, 11,785 square feet of BL-2 laboratory space (with additional support areas), and a 7,500 square foot BL-3 animal care facility. This BL-3 space will directly support DIR's SIV research effort. The initial planning of the Phase 1A renovation which includes provisions for a library, seminar room, offices and lunch room in an aesthetically pleasing and productive work environment, began in July of this fiscal year. This renovation is scheduled for completion in July 1991.

The need for additional laboratory space for AIDS research has increased dramatically as the AIDS epidemic has grown. This has led to the approval of a new A wing addition to Building 10. This facility is dedicated and designed specifically to meet the requirements of AIDS research. Construction has begun on the new 10A Wing and the AMB is gearing up for movement of administrative and laboratory staff scheduled for occupancy in July 1991. DIR will consume approximately 8,700 square feet of space on the 4th and 6th floors of this building. This space will be divided among the Laboratory of Immunoregulation, the Laboratory of Molecular Microbiology (6,500 square feet on the 6th floor), the Office of the Director, and the Office of the Chief, AMB (2,200 square feet on the 4th floor).

Other space relocations and/or renovations occurring this fiscal year include the relocation of the ACRF, 11th floor AIDS Clinic to the 8th floor in June of this year. The AIDS Clinic previously occupied 1,975 square feet of space and now operates with increased space totalling 3,900 square feet. AMB personnel have worked closely with the NCI contractor, PRI, to develop plans for the Transgenic Mouse facility in Building 550, Frederick. Renovations to Building 550 to house this facility are planned for the Fall of 1990. This facility will also require additional storage space and AMB has asked PRI to perform a feasibility study regarding the possibility of constructing an addition to the building.

Procurement/Contracts

In FY '90, AMB monitored and managed a contract portfolio, totalling $20 million consisting of 17 research and development contracts including PRI, (which has now expanded to 15 individual projects), 10 procurement contracts, as well as several interagency agreements. The contract status and budget tracking reports were upgraded to provide more detailed information specifically for renewals and Board Reviews. They were designed to work in concert with the SOF and AMBIS reports to provide easy monitoring of all aspects of the DIR contracting process. The procedures for the renewal of expiring contracts and the establishment of new contracts, as well as the format of the Directors Annual Contract Review were further formalized and communiques more standardized to insure DIR laboratories are fully supported and informed in all phases of the contracting process.

All AMB Offices were audited this year by the Division of Financial Management to determine whether adequate documentation exists to support obligation and receipt transactions that were recorded through the DELPRO. In order to insure preparedness for these reviews, NIAID established its own Procurement Review Committee that was charged with the responsibility of conducting similar audit scenarios. This review process has been instrumental in AMB's successful audits. In addition, all AMB offices were audited by the Division of Procurement to review acquisition files. The results of these reviews indicated that AMB is in full compliance with established procurement and financial management policies and procedures. This is indeed an accomplishment particularly since the DIR continues to lead NIH in the number of transactions per purchasing agent.

Hurricane Hugo hit the Caribbean Primate Center in September of 1989. Extensive damage was caused to many portions of the Center but no physical harm was found to the NIAID breeding colony of animals. Due to the urgency of this situation, the administrative staff responded quickly and was able to engineer the cooperation of various NIH Divisions to ensure the prompt purchase of emergency equipment and supplies. The DIR also implemented a Procurement Improvement Committee, chaired by the Chief AMB and made up of representatives from all labs. This was done to provide an ongoing forum for lab staff to direct procurement issues which require clarification, improvement or cause concern. The members have requested that it continue and feel it is a valuable committee where important information is converged and exchanged.

Automation

DIR automation support continues to be provided through contract and from NIAID's Information Technology and Evaluation Branch (ITEB). The DIR currently has over 650 LAN users. The Administrative Management and Budget Information System (the only one of its kind in the NIH) was implemented in FY '89. This system streamlines the procurement process for scientists, by providing guidelines for placing orders according to appropriate regulations, prompt status reports on each request, automated budget tracking and access to the NIH BPA listings. This system has provided purchasing agents with more time to place orders and less time on budget tracking. AMBIS also reconciles NIH's Division of Financial Management reports with AMBIS and DELPRO fiscal data.

An overview of AMBIS has been presented to NIH Executive Officers and Intramural Administrative Officers. Other ICDs have shown interest but due to their lack of a wide area network are unable to benefit from AMBIS (at this time). Additionally, The Division of Procurement is also reviewing AMBIS to assess its capabilities as an NIH-wide system. AMBIS's design, which supports proper procurement practices, is a key element in NIH's review. This fiscal year AMBIS underwent a number of enhancements and was installed in all Administrative Offices and laboratories except the Rocky Mountain laboratories.

In an effort to maintain better tracking of clinical trials and protocols, particularly for AIDS patients, a clinical program database was created to look at all clinical studies and trials. This program is a very useful tool for determining the cost of each active clinical protocol from inception to completion. This system also allows us to better monitor and control clinical expenditures.

Personnel

The responsibilities placed upon the AMB during the last 5 years have become immense in the areas of procurement, personnel, contracts, budget, patent policy, material transfer, etc. As a result, the AMB began the new year under a different organizational structure. On December 5, 1989, AMB's reorganization request was approved. The reorganization was implemented on January 22, 1990. This reorganization established AMB into two operational sections within AMB. The sections include the Financial Management Services Section which is responsible for contract administration, financial management and budget execution, clinical research management, management analysis, automated systems and information planning. The Operations Support Services Section is responsible for personnel, budget administration, procurement, program analysis, and day-to-day administrative support to DIR. In addition, the Chief, AMB, is functioning in a dual capacity of Chief, AMB, DIR and Chief of the newly established Technology Transfer Branch, OAM, OD, NIAID. This new Branch is responsible for technology transfer coordination activities and acquisitions oversight. AMB's current structure is now being used as a model for other ICD's when organizing their administrative offices.

In an effort to further encourage cooperation and understanding between our investigators and the administrative staff, the AMB instituted a series of seminars that are provided by DIR Laboratory Chiefs and Sections Heads. These seminars provide the scientist with a forum to discuss their research and allow the administrative staff an opportunity to ask questions concerning the scientist's area of research. These seminars have fostered better lines of communication between the purchasing agents, administrative and laboratory staffs.

The AMB assisted in the establishment of the Molecular Allergy and Immunology Section, which is organizationally located within the Office of the Scientific Director. The Branch assisted in staffing and other personnel matters, budget tracking and ordering new equipment for this new laboratory. The AMB played a critical role in establishing other DIR organizational changes, including the establishment of the Laboratory of Intracellular Parasites, combining the Microbiology and Immunology Section from the OSD to the Laboratory of Immunogenetics, etc.

Earlier in the fiscal year, it was determined that the DIR could save a considerable amount of money by transferring many of its contracts in-house. AMB has worked on reviewing salary issues, benefits, etc., and is in the process of converting employees working in these contracts to civil service or staff fellow positions. Contract conversions will continue during the next fiscal year.

The AMB implemented a number of internal procedures this fiscal year for handling new personnel mandates such as the AIDS Loan Repayment Program, Incentive Special Pay for Commissioned Officers, Senior Staff Fellow pay increases, the PreIRTA Program, etc. Other AMB personnel initiatives included revamping Promotion Advisory Committee review procedures and establishing new property management guidelines. Special projects that the AMB successfully completed included routinely providing an analysis of DIR's overtime usage and approval, revising supervisory EPMS elements and PMRS plans, implementing action items (e.g.. technicians traveling to meetings to present abstracts) suggested by DIR technicians in their meeting with the Director, DIR, adding more generic elements to AMBIS, etc. This year the AMB also placed particular emphasis on streamlining administrative procedures such as the Annual Personnel Review and Organization Reviews, and the review process for personnel actions and outside activities.

This fiscal year, the AMB actively participated in three Board of Scientific Counselors (BSC) Reviews for the Laboratory of Molecular Microbiology (LMM), the Laboratory of Viral Diseases (LVD) and the Laboratory of Clinical Investigation (LCI). This was the first year that the AMB prepared a separate administrative section for these reviews. The administrative section included contract review summaries, personnel listings by section and job classification, expenditure (budget) reports, space reports, floor plans, etc. The BSC Reviews were very successful and the administrative staff received exceptionally laudatory remarks from the LCI Review.

BIOLOGICAL RESOURCES BRANCH
1990 Annual Report
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Annual Report
Biological Resources Branch
National Institute of Allergy and Infectious Diseases
October, 1, 1989 to September 30. 1990

RESEARCH PROGRESS

The Biological Resources Branch investigates the structure and function of the genes and molecules involved in the immune response, the molecular basis for antigen presentation and recognition, and hemapoietic cell differentiation. In addition to this fundamental immunological research, the Branch provides protein sequencing, peptide synthesis and flow cytometry expertise and services for the NIAID.

STUDIES OF THE MAJOR HISTOCOMPATIBILITY COMPLEX

Major histocompatibility complex (MHC) encoded class I molecules are integrally involved in the presentation of viral antigens, in the form of processed peptides, to cytotoxic T lymphocytes (CTL). Studies on the molecular basis of viral antigen processing and the nature of the interaction of processed antigen (peptides) with class I molecules, as well as factors controlling the expression of class I and class II molecules have been emphasized in this research.

Peptide Binding Specificity of Class I Molecules. The ability of HLA-A2 and HLA-B37 molecules to bind a set of randomly selected peptides was studied in order to learn more about the specificity of the peptide binding site in class I molecules. It was shown that: 1) the HLA-A2.1 molecule can bind a broad spectrum of peptides; and 2) T cells selected for the ability to recognize one peptide plus a class I molecule can actually recognize an unrelated peptide presented by the same class I molecule. To determine if another class I molecule would bind the same or a different set of peptides, we studied the binding specificity of HLA-B37 with the same set of peptides. We examined the capacity of this panel of peptides to compete for the presentation of influenza virus nucleoprotein peptide NP-(335-350) by HLA-B37 to NP-peptide-specific HLA-B37-restricted cytotoxic T-lymphocyte lines. Forty-two percent of peptides tested were capable of inhibiting NP-(335-350) presentation by HLA-B37. Remarkably, none of these HLA-B37 binding peptides belong to the subset that was previously shown to bind to the HLA-A2.1 molecule. Only the NP (335-350) peptide was capable of binding to both HLA-A2.1 and HLA-B37. These findings demonstrate that the peptide-binding specificities of HLA-B37 and HLA-A2.1 are largely nonoverlapping and suggest that, from the universe of peptides, individual HLA class I molecules can bind to clearly distinct subsets of these peptides.

Class I Binding Site Residues Important for Peptide Presentation. Amino acid substitutions were made at key sites in the β-pleated floor of the HLA-A2 peptide binding groove in order to study their effect on recognition by allospecific and peptide specific CTL. Recognition by allospecific CTL was generally unaffected by substitutions at positions 9, 43, 95 or 156. In contrast, recognition by HLA-A2.1 restricted influenza matrix peptide specific CTL was totally eliminated by single amino acid substitutions at positions 9 or 156. The ability of amino acid side chains in the floor of the peptide binding groove of HLA-A2 to affect the presentation of a viral peptide to peptide-specific cytotoxic T lymphocytes (CTL) was examined further using HLA-A2 molecules with naturally occurring single amino acid substitutions. Substitutions in the HLA-A2 molecule of a Phe to Tyr at position 9 (Tyr9), a Tyr to Cys at position 99 (Cys99) and a Val to Leu at position 95 (Leu95) were examined for their ability to present the influenza virus matrix M1 55-73 peptide and several sequence variants of the M1 peptide to a panel of 36 M1 peptide-specific HLA-A2.1-restricted CTL lines. The results obtained demonstrated that amino acid side chains in the floor of the peptide binding groove can have a major functional role in determining the formation and conformation of the peptide-A2 complex that is recognized by allospecific and peptide-specific CTL.

Class I Binding Site Residues Important for T Cell Repertoire Selection. Two different HLA-A2 genes were found to be expressed in cells from donor Q66; one is identical to HLA-A2.1 and the other is identical to HLA-A2.2F (Gln → Arg at position 43, Val → Leu at position 95, and Leu → Trp at position 156). Studies with CTL generated from this donor demonstrated that a different CTL repertoier specific for HLA-A2 plus the M1 55-73 peptide is generated in an individual that expresses both HLA-A2.1 and HLA-A2.2F compared to individuals who express HLA-A2.1 alone, and that the unique repertoire of T cell receptors can be selected by the presence of an HLA-A2 molecule with a single amino acid substitution at position 156.

Allorecogntion of Class I Molecules. To further investigate the role of α_2 helical region in the recognition of H-2K^b, the effects of peptide K^b163-174 on allospecific T-cell lines and clones were studied. The results indicated that peptide K^b163-174 interferes with T-cell receptor engagement of a contact site on the H-2K^b molecule. It was concluded that, amino acid residues 163-174 define a site used by many alloreactive T cells to engage the H-2K^b molecule. The TrAH10 monoclonal antibody is unique among conventional HLA typing reagents in that it is able to recognize lymphoid cells that express HLA-A*0301, but not those that express HLA-A*0302. Analysis of the specificity of this antibody has demonstrated that it binds to an epitope that is located between amino acids 144-160 on the α_2 helix. Several lines of evidence indicated that the Arg at position 145 and the Glu at position 152 are critical for the formation of the epitope.

Novel Class I Molecules. A novel mouse H-2 class I gene, named "37", having a molecular size of 45 kD was found to be expressed in a variety of tissues indicating that class I molecules other than K, D and L may play a role in immune function. This mouse gene, encoded in the Tla region, is not very polymorphic and is expressed by all mouse strains examined except A/J mice.

Diversity of γ/δ T Cell Receptors. An anti-T cell receptor (TCR) γ-chain variable region serum was generated and used to more precisely define the TCR γ chains expressed at the protein level in γ/δ TCRs, this serum allowed for the initial identification of the expression of a new type of TCR γ chain, namely a $V\gamma1.2$ - $C\gamma2$ chain. In addition, use of this antiserum showed that the molecular size diversity in $V\gamma1$-$C\gamma4$ containing γ/δ TCRs was due mainly to the diversity in the δ chains. As a first approach to analyzing the genetic influences on the TCR $\gamma\delta$ repertoire, $\gamma\delta$ TCR heterodimers expressed in the spleens of different mouse strains were examined. All strains of mice studied could be categorized into one of three basic phenotypes. These phenotypes were not determined by genetic influences, but by polymorphic differences in the TCR γ genes.

T Cell Receptors in Human Skin. The epidermis of human skin harbors a phenotypically heterogeneous population of T lymphocytes (TCs). The majority of these are $CD2^+/CD3^+/CD5^+$ "memory" cells exist in an unactivated state and express the α/β TCR. In contrast to murine skin, only a very minor subpopulation of $CD3^+$ cells in the human epidermis bear the γ/δ TCR. Epidermal TCs primarily are distributed along the rete ridges in the basal keratinocyte layer and are often in close apposition to Langerhans cells (LCs). Epidermal TCs were significantly ($p<0.0001$) more abundant in the sole than in the other body regions examined (i.e., 40 vs. 7 $CD3^+$ cells/linear centimeter of epidermis) and seemed to have a particular affinity for the acrosyringial epithelium of eccrine sweat ducts. Moreover, in most areas and particularly the sole, there was a significantly higher percentage of $CD8^+$ vs $CD4^+$ cells. This study not only provided evidence of significant regional variability in the human epidermal TC population of normal skin, but also strengthened the concept for skin-associated lymphoid tissues, whereby memory TCs recirculate back to the epidermis and interact with resident antigen-presenting cells (i.e., LC).

Assembly of T Cell Receptors. Cell surface-expressed receptors are often multi-chain complexes. One of these, the T cell receptor α/β-CD3 complex, is known to contain at least seven chains: the α and β TCR chains plus the γ, δ, ε and two ζ chains from the CD3 complex ($\alpha\beta\gamma\delta\varepsilon\zeta_2$). To gain insight into the structure of the complex, anti-peptide sera specific for individual subunits of the complex were used to determine subunit interactions within the complex. Four closely associated pairs of chains could be identified: $\alpha\beta$, ζ_2, $\gamma\varepsilon$ and $\delta\varepsilon$. Interactions between the TCR $\alpha\beta$ and either $\gamma\varepsilon$ or $\delta\varepsilon$ could be observed in the apparent absence of other CD3 chains. Furthermore, a hierarchy in the strength of the association between the TCR and the individual CD3 chains could be distinguished: TCR ε > TCR δ > TCR γ. The ζ_2 dimer could only be detected in "intact" TCR-CD3 complexes. Finally, cross-linking experiments indicated a close spatial relationship between the TCR $\alpha\beta$ and both the CD3-γ and CD3-ε chains. Based on the observations a model for the structure of the TCR-CD3 was proposed.

SYNTHESIS OF PEPTIDE ANTIGENS

During the last year over 400 peptides have been produced by a PRI contract facility managed by the Branch and within the Branch itself. These peptides have supported research projects in nearly all Laboratories of the NIAID. Synthetic peptides have been used to define and study cytotoxic T lymphocyte or helper T cell epitopes for HIV, rotavirus, influenza virus, chlamydia, ovalbumin, myelin basic protein and heat shock proteins. Synthetic peptides have been used to prepare antisera against proteins originally described by protein or nucleic acid sequence analysis. Such antisera have been prepared against the proteins form the following sources: vaccinia virus, respiratory syncytial virus, MAIDS virus, cowpox virus, dengue virus, HIV, Plasmodium falciparum, rat cyclophilin/PPIase, T cell activation proteins, MHC class II DR and DP molecules, B cell specific proteins and Fcε receptors. In addition, peptides have been prepared to study chemotaxis and to mimic the activity of TAT form the HIV virus.

Peptides have been synthesize by members of the BRB for a variety of research projects. Such peptides have been used to prepare antisera to the α and β chains of integrins in order to study lymphocyte activation, interaction and trafficking. This has allowed the identification of the vitronection receptor (VNR) as a T cell activation antigen. By making antisera to a cDNA sequence, we have identified a protein encoded by the SCL locus that seems to play a fundamental role in hemapoietic differentiation. Peptides derived from HTLV-I have been used to define antibody reactivities in patients sera and to develop a very specific screening assay for discriminating HTLV-I and HTLV-II infections. Peptides have been used to define the nature of the peptide binding site of the HLA-A2 and -B37 molecules and to define influenza specific T cell epitopes in certain mouse strains. A detailed study was performed on the dissection of H-2D^{b} -restricted cytotoxic T-lymphocyte epitopes on simian virus 40 T antigen by the use of synthetic peptides and H-2D^{bm} mutants.

PROTEIN SEQUENCE ANALYSES

The facility has analyzed about 300 samples during the past nine months and projects that it will analyze about 400 samples this fiscal year from the following sources:

NIAID	Sample No.
BRB	285 (peptide quality control)
LVD	77
LI	2
LIR	2
LMSF	4
NCI	11
Extramural	6
Total	387

Proteins from a large variety of sources have been sequenced in the facility. These include TGFβ-related proteins (A. Roberts, NCI); vaccinia virus polymerases (P. Gershon and B. Ahn, LVD); HSVI polymerase fractions (M. Chalberg, LVD); a vaccinia membrane protease (J. Keck, LVD); tumor specific antigens (B. Seon, Roswell Park); lymphocyte activation antigens (M. Sitkovsky, LI); and a serum class I associated protein (D. Margulies, LI).

FLOW CYTOMETRY ANALYSIS

Over the past year the FCS has accomplished the following: The FCS computers are now connected, via ethernet (using LAN cabling), to both IBM PC-compatible and MacIntosh PC's for analysis of data from Buildings 4,7 and 10. A new MicroVax 3000 with two large capacity disk drives, has been installed which serves as the network router and will also serve as a data analysis station. PC based analysis software, including a Coulter workstation, are being evaluated for analysis of data from all FCS instruments. The computer processor of the user operated Epics Profile has been upgraded to greatly enhance analysis and acquisition of data.

Biological Resources Branch
Annual Report
October 1, 1989 to September 30, 1990

ADMINISTRATIVE REPORT

This period marks the third year of the existence of the Biological Resources Branch. There are four Sections within the Branch; three of which are primarily involved with providing technological resources to the NIAID intramural laboratories. In the Molecular Structure Section, Mark Garfield continues to be responsible for protein sequence analyses and during the past year has taken on the additional responsibility of making about 25% of the synthetic peptides produced within the Branch. The Flow Cytometry Section continues to flourish under the able direction of Dr. Kevin Holmes and Mr. David Stephany. The turmoil in the production of synthetic peptides created by the departure of Dr. W. Lee Maloy last year has subsided and the Branch produced a record number of peptides during the past year. Dr. Coligan assumed overall responsibility for the production of peptides both by the Branch itself and by the PRI contract.

On the research side, Dr. Ken Parker has been hired as a Senior Staff Fellow in the Synthetic Peptide Antigen Section to work on problems in structural biology. In particular, his immediate interest center around defining the physico-chemical interaction between MHC class I molecules and peptide. A new technician, Douglas Markert, hired for the Section has since departed and recruitment is underway. In regard to postdoctoral fellows, Dr. Gregory Einhorn has departed for a position at the Research Institute of Scripps Clinic, Dr. Thomas McConnell has assumed an Assistant Professorship at East Carolina University and Dr. Kevin Moulder departed for a position at Smith Kline Beecham, Epsom, England. Dr. Antonello Punterieri joined the laboratory as a Visiting Fellow from Dr. Hiroshi Taniuchi's lab in the NIDDK, Dr. Mark Halvorson has arrived from the Department of Microbiology, Indiana University to assume an IRTA position, and Dr. Chiara Bovolenta joined the laboratory as a Guest Researcher from the University of Verona, Italy.

HONORS AND AWARDS

Dr. Coligan was invited to present laboratory data seminars at the Center for Disease Control in Atlanta and the University of Leiden in Holland. He was also invited to make presentations at the MHC Workshop in Bavaria, West Germany, the FASEB meeting in New Orleans and the Workshop in Molecular Biology of MHC Genes in Italy. Dr. Kevin Holmes made an in invited presentation at the UCLA symposium on B-cell development. Dr. Coligan continues to serve on the editorial boards of Molecular Immunology and Immunologic Research and during the past year was asked to serve on the editorial broad of Current Protocols in Immunology. Dr. Coligan was appointed to the Senior Executive Service. He was also appointed as Chairperson on the Program Committee for the Major Histocompatibility Complex Section of the American Association of Immunologists Annual Meeting.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00169-13 BRB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Murine and Human Transplantation Antigens and Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John E. Coligan Branch Chief BRB, NIAID

COOPERATING UNITS (if any)

P. Kourilsky, Pasteur Institute; E. Long, LIG, NIAID, W. Biddison, NI, NINCDS, D. Margulies, LI, NIAID.

LAB/BRANCH

Biological Resources Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
1.2	.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two different HLA-A2 genes were found to be expressed in cells from donor Q66; one is identical to HLA-A2.1 and the other is identical to HLA-A2.2F (Gln → Arg at position 43, Val → Leu at position 95, and Leu → Trp at position 156). Studies with CTL generated from this donor demonstrated that a different CTL repertoire specific for HLA-A2 plus the M1 55-73 peptide is generated in an individual that expresses both HLA-A2.1 and HLA-A2.2F compared to individuals who express HLA-A2.1 alone, and that the unique repertoire of T cell receptors can be selected by the presence of an HLA-A2 molecule with a single amino acid substitution at position 156. A peptide derived from α_2 helical region of the H-2K^b molecule, peptide K^{b}163-714, was previously shown to specifically inhibit the stimulation of an alloreactive T-cell hybridoma. To further investigate the role of this region in the recognition of H-2K^b, the effects of peptide K^{b}163-174 on allospecific T-cell lines and clones were studied. The results indicated that peptide K^{b}163-174 interferes with T-cell receptor engagement of a contact site on the H-2K^b molecule. It was concluded that amino acid residues 163-174 define a site used by many alloreactive T cells to engage the H-2K^b molecule. The TrAH10 monoclonal antibody is unique among conventional HLA typing reagents in that it is able to recognize lymphoid cells that express HLA-A*0301, but not those that express HLA-A*0302. Analysis of the specificity of this antibody has demonstrated that it binds to an epitope that is located between amino acids 144-160 on the α_2 helix. Several lines of evidence indicated that the Arg at position 145 and the Glu at position 152 are critical for the formation of the epitope. A novel mouse H-2 class I gene, named "37", having a molecular size of 45 kD was found to be expressed in a variety of tissues indicating that class I molecules other than K, D and L may play a role in immune function. This mouse gene, encoded in the Tla region, is not very polymorphic and is expressed by all mouse strains examined except A/J mice. Primary structural characteristics important for the pairing of the α and β chains of human class II DR and DP molecules were defined.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI-00352-08 BRB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Characterization of Cell Surface Molecules Important for Immune Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	John E. Coligan	Branch Chief	BRB/NIAID
OTHERS:	Angel Ezquerra	Visiting Fellow	BRB/NIAID
	Kevin Moulder	Visiting Fellow	BRB/NIAID
	Gary Kikuchi	NRC	BRB/NIAID
	Tom McConnell	NRC	BRB/NIAID
	Scott Wadsworth	IRTA	BRB/NIAID
	Gregory Einhorn	IRTA	BRB/NIAID
	Antonello Punterieri	Visiting Fellow	BRB/NIAID

COOPERATING UNITS (if any)
Georg Stingl, Vienna Univ., Austria; Jeff Bluestone, Univ. of Chicago; Ethan Shevach, LI, NIAID.

LAB/BRANCH
Biological Resources Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6.4	5.7	.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An anti-T cell receptor (TCR) γ chain variable region serum was generated and used to more precisely define the TCR γ chains expressed at the protein level in γ/δ TCRs, this serum allowed for the initial identification of the expression of a new type of TCR γ chain, namely a Vγ1.2 - Cγ2 chain. In addition, use of this antiserum showed that the molecular size diversity in Vγ1-Cγ4 containing γ/δ TCRs was due mainly to the diversity in the δ chains. As a first approach to analyzing the genetic influences on the TCR $\gamma\delta$ repertoire, $\gamma\delta$ TCR heterodimers expressed in the spleens of different mouse strains were examined. All strains of mice studied could be categorized into one of three basic phenotypes. These phenotypes were not determined by genetic influences, but by polymorphic differences in the TCR γ genes.

Human skin was found to differ from mouse skin by the fact that it contains very few γ/δ TCR expressing cells. Most CD3$^+$ cells were α/β TCR$^+$, CD8$^+$. Regions of the epidermis were found to vary significantly in the percentage of TCR bearing cells with the acrosyringial epithelium of eccrine sweat ducts having a high abundance of such cells.

The α/β TCR-CD3 complex is known to contain at least seven chains ($\alpha\beta\gamma\delta\varepsilon\zeta_2$). Using anti peptide serum to precipitate complexes under various conditions of dissociation as well as after crosslinking. Four closely associated pairs of chains could be identified: $\alpha\beta$, ζ_2, $\gamma\varepsilon$ and $\delta\varepsilon$. Interaction between the TCR $\alpha\beta$ and either $\gamma\varepsilon$ or $\delta\varepsilon$ could be observed in the apparent absence of other CD3 chains. Furthermore, a hierarchy in the strength of the association between the TCR and the individual CD3 chains could be distinguished: TCR ε > TCR δ > TCR γ. The ζ_2 dimer could only be detected in "intact" TCR-CD3 complexes. Finally, cross-linking experiments indicated a close spatial relationship between the TCR $\alpha\beta$ and both the CD3-γ and CD3-ε chains. Based on the observations a model for the structure of the TCR-CD3 was proposed.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AI 00543-03 BRB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Recognition of Peptidic Antigens by Virus-Specific Cytotoxic T Lymphocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	John E. Coligan	Chief	BRB, NIAID
OTHERS:	Robert W. Anderson	Staff Fellow	BRB, NIAID
	Kenneth C. Parker	Senior Staff Fellow	BRB, NIAID

COOPERATING UNITS (if any): Bill Biddison, NI, NINCDS.

LAB/BRANCH: Biological Resources Branch

SECTION: Office of the Chief

INSTITUTE AND LOCATION: NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.4	1.4	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The ability of HLA-A2 and HLA-B37 molecules to bind a set of randomly selected peptides was studied in order to learn more about the specificity of the peptide binding site in class I molecules. It was shown that: 1) the HLA-A2.1 molecule can bind a broad spectrum of peptides; and 2) T cells selected for the ability to recognize one peptide plus a class I molecule can actually recognize an unrelated peptide presented by the same class I molecule. To determine if another class I molecule would bind the same or a different set of peptides, we studied the binding specificity of HLA-B37 with the same set of peptides. We examined the capacity of this panel of peptides to compete for the presentation of influenza virus nucleoprotein peptide NP-(335-350) by HLA-B37 to NP-peptide-specific HLA-B37-restricted cytotoxic T-lymphocyte lines. Forty-two percent of peptides tested were capable of inhibiting NP-(335-350) presentation by HLA-B37. Remarkably, none of these HLA-B37 binding peptides belong to the subset that was previously shown to bind to the HLA-A2.1 molecule. Only the NP (335-350) peptide was capable of binding to both HLA-A2.1 and HLA-B37. These findings demonstrate that the peptide-binding specificities of HLA-B37 and HLA-A2.1 are largely nonoverlapping and suggest that, from the universe of peptides, individual HLA class I molecules can bind to clearly distinct subsets of these peptides.

Amino acid substitutions were made at key sites in the β-pleated floor of the HLA-A2 peptide binding groove in order to study their effect on recognition by allospecific and peptide specific CTL. The results obtained demonstrated that amino acid side chains in the floor of the peptide binding groove can have a major functional role in determining the formation and conformation of the peptide-A2 complex that is recognized by peptide-specific CTL.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00523-03 BRB

PERIOD COVERED October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Flow Cytometry Section

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kevin Lee Holmes Senior Staff Fellow BRB, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH Biological Resources Branch

SECTION Office of the Chief

INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
5.25	.25	5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The linking of the DIR PCs, both IBM compatibles and MacIntoshes, via the LAN, to FCS computers has been successfully implemented. Users in buildings 4,7 and 10 are utilizing the networking services to analyze data acquired on all three multiparameter flow cytometers. Commercially available PC-based analysis software is being evaluated for incorporation into the analysis capabilities of the FCS, including the installation of a Coulter analysis workstation. The overall usage of FCS instrumentation has been approximately equal to FY '89, totaling 2954 hrs or 3 hrs/day/instrument. This represents an effective use of instrument time over FY '90.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00172-12 BRB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Synthesis of Peptide Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	John E. Coligan	Branch Chief	BRB, NIAID
Others:	Robert Anderson	Staff Fellow	BRB, NIAID

COOPERATING UNITS (if any)
Thomas Folks, (CDC); Ilan Kirsch, NCI.

LAB/BRANCH
Biological Resources Branch

SECTION
Synthetic Peptide Antigen Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.3	0.6	.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the last year over 400 peptides have been produced by a PRI contract facility managed by the Branch and within the Branch itself. These peptides have supported research projects in nearly all Laboratories of the NIAID. Synthetic peptides have been used to define and study cytotoxic T lymphocyte or helper T cell epitopes for HIV, rotavirus, influenza virus, chlamydia, ovalbumin, myelin basic protein and heat shock proteins. Synthetic peptides have been used to prepare antisera against proteins originally described by protein or nucleic acid sequence analysis. Such antisera have been prepared against the proteins from the following sources: vaccinia virus, respiratory syncytial virus, MAIDS virus, cowpox virus, dengue virus, HIV, Plasmodium falciparum, rat cyclophilin/PPIase, T cell activation proteins, MHC class II DR and DP molecules, and Fcε receptors. In addition, peptides have been prepared to study chemotaxis and to mimic the activity of TAT from the HIV virus.

Within the Branch, synthetic peptides were used to map the H-2 restricted cytotoxic T-lymphocyte epitopes on Simian Virus 40 (SV40) T antigen. Five distinct CTL recognition sites were identified in the SV40 T antigen, four of these were $H\text{-}2D^b$ restricted and one was $H\text{-}2K^b$ restricted. Three of the four $H\text{-}2D^b$ sites were clustered in the amino terminal half of the large T antigen. Evidence was obtained indicating that the synthetic peptides did not completely mimic the activity of endogenously processed large T antigen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00522-03 BRB

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Protein Sequence Analyses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John E. Coligan Branch Chief BRB, NIAID

COOPERATING UNITS (if any)
A. Roberts and M. Sporn, NCI; J. Sachs, London School of Hygiene and Tropical Medicine; B. Seon, Roswell Park Memorial Institute; P. Gershon, LVD, NIAID; D. Margulies, LI, NIAID, H. Slayter, Dana Farber Cancer Institute.

LAB/BRANCH
Biological Resources Branch

SECTION
Molecular Structure Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
.7	.1	.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues X ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the past year, the amino acid sequences of over 295 synthetic peptides and the N-terminal sequences of 119 protein were determined by automated protein sequence analysis. In several cases N-terminally blocked proteins were treated with CNBr prior to sequence analysis in order to generate unblocked N-termini. The synthetic peptides were analyzed as a means of quality control of the peptides produced by the NIAID peptide synthesis facilities. In some cases, tryptic peptides derived from proteins were isolated by reverse phase HPLC and their sequences were determined. Some of the studies impacted by this work concern: 1) MHC class II associated protein (London School of Hygiene and Tropical Medicine); tumor specific antigens (Roswell Park Memorial Inst.); vaccinia and Herpes Simplex virus proteins (Laboratory of Viral Diseases); TGFβ and recombinant TGFβ (NCI); vaccinia encoded proteins (Laboratory of Viral Diseases); a serum protein associated with H-2 class I molecules and lymphocyte activation proteins (Laboratory of Immunology).

Laboratory of Cellular and Molecular Immunology
1990 Annual Report
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PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Cellular and Molecular Immunology
October 1, 1989 through September 30, 1990

Introduction

The Laboratory of Cellular and Molecular Immunology was created in November, 1986. Its major task is to perform research on thymus-derived (T) lymphocytes. Its objective is to understand how these critical cells of the immune system differentiate and function. The approach is to define problems in whole animal systems, set up *in vitro* analogs in tissue culture, and determine the molecular basis for the phenomena. The Laboratory employs techniques from the disciplines of cellular immunology, molecular biology, and protein biochemistry.

During its fourth year, the Laboratory has pursued four major areas of research: *1)* antigen presentation by resting B cells, *2)* the biochemical basis of T cell inactivation, *3)* the intrathymic events in T cell differentiation, and *4)* a new *in vitro* assay for cytotoxic T cells.

Antigen Processing and Presentation

The antigen-presenting cell controls the outcome of T cell antigen-receptor occupancy by its ability to delivery a costimulatory signal. In the presence of costimulation, the T cell makes interleukin 2 (IL-2) and divides. In the absence of this second signal, the T cell makes only a partial response and is induced into a nonresponsive state known as clonal anergy. Thus, different cells types bearing major histocompatibility complex (MHC) molecules and antigen will have differential effects on the immune response, depending on their ability to delivery the costimulatory signal. During the past year, we have investigated the ability of small, low density ("resting") B cells to present antigen to T cells *in vivo* and *in vitro*. *In vitro*, these cells stimulate a proliferative response from T cell clones, although this capacity is abolished by pre-irradiating the B cells with 3000R. This observation suggested that the B cells express costimulatory activity. In contrast, the same population, when injected *in vivo*, induced partial or complete tolerance in two systems, the cytotoxic response to the male antigen H-Y and the mixed lymphocyte response in full MHC incompatible stimulation. These observations suggested that resting B cells do not express costimulatory activity. This paradox was resolved when it was discovered that overnight incubation of resting B cells in tissue culture made them radioresistant for antigen presentation. This suggested that resting B cells lack costimulatory activity, but that it is induced during *in vitro* culture. This induction process appears to be sensitive to 3000R irradiation.

(E. Fuchs, H. Hashimoto, A. Bendelac, C. Prussin, and R. H. Schwartz, LCMI, NIAID)

The Biochemical Basis of T Cell Activation and Inactivation

Studies of T cell activation of normal murine T cell clones of the T_H1 type have shown that two signals are required to stimulate the cells to make IL-2 and divide. One signal is given through the antigen-specific receptor that is uniquely expressed on each clone. The other signal is called costimulation and is delivered through a different receptor that has not yet been characterized. Signal 1 in the absence of signal 2 induces a state of hyporesponsiveness in the T cell clone known as clonal anergy. In this state, the cell fails to make significant amounts of IL-2 when stimulated with both signals 1 and 2. During the past year, the reasons for this failure to make IL-2 were examined at the molecular level. Although signaling through the antigen receptor was found to be normal, as assessed by increases in intracellular calcium and protein kinase C activation, steady-state levels of IL-2 mRNA were decreased 20- to 50-fold. Other lymphokines were also affected, but not as profoundly. Interleukin 3 production in response to stimulation was down 8-fold, and γ-interferon and tumor necrosis factor production were down 3-fold.

In an attempt to extend our observations on T_H1 clones to other T cell subpopulations, we tried to induce clonal anergy in T_H2, T_H0, and freshly isolated T lymphocytes from immunized mice. Three *bona fide* T_H2 clones could not be anergized with any of three techniques used to anergize T_H1 clones. In contrast, two T_H0 clones could be anergized. The common theme appears to be whether the T cell clone depends on IL-2 for its autocrine growth. T_H1 and T_H0 clones, which utilize IL-2, can be anergized, whereas T_H2 cells, which utilize different growth factors, cannot. Finally, freshly isolated T cells behaved in an anomalous manner. These cells could be anergized when exposed to the calcium ionophore, ionomycin, but not when stimulated with antigen on chemically-fixed presenting cells. The reason(s) for this last paradoxical set of results is still under investigation.

(D. Mueller, B. Beverly, S. Stringfellow, K. Brorson, D. Cassell, K. Inamori, and R. H. Schwartz, LCMI, NIAID; S. Kang and M. Lenardo, LI, NIAID)

T Cell Differentiation

This project is aimed at defining and characterizing the series of developmental events involved in the generation of mature, immunocompetent T cells and the selective events that determine the antigen-recognition repertoire of these cells. These studies primarily utilize the thymus, the major site of T cell differentiation in the animal. In the past, we have identified a number of thymocyte cell types and made possible the isolation of these cells at very high purity on a preparative scale. We have used these isolated cell types in an effort to define the state of maturation, precursor-product and lineage relationships, expression of T cell receptors (TCRs), and the mechanisms and inductive signals responsible for growth, differentiation, and selection in the thymus.

During the past two years, we have been analyzing radiation-induced bone marrow chimeras for the development of tolerance to self antigens. In particular, we have asked whether the thymic epithelial cells can contribute to this process. Using the Mls antigen,

we have demonstrated that epithelial cells do not induce clonal deletion. Instead, they impart a type of clonal anergy in which the T cells still exist, but can no longer be activated to divide by stimulation with anti-receptor antibodies or the Mls antigen *in vitro*. In this system, the thymic epithelial cells do not synthesize the Mls antigen, but instead pick it up from other cells in the irradiated hosts that are presumably shedding it. Other experiments have demonstrated that both $CD4^+$ and $CD8^+$ thymocytes are anergized, even though the Mls molecules under study only function to stimulate through interaction with MHC class II molecules. While these experiments suggest that anergy induction occurs at the $CD4^+8^+$ double-positive stage in thymocyte development, an attempt to support that hypothesis by *in vivo* anti-CD4 treatment did not give the expected result, *i.e.*, blocking of anergy induction in $CD8^+$, $V\beta17^+$ T cells did not occur. Thus, either anergy induction does not occur at the $CD4^+8^+$ stage, or the CD4 molecule is not involved in the interactions that lead to this form of tolerance.

In our previous efforts to define the maturational stages of T cell development, we showed that $CD4^+8^-$ thymocytes could be subseted with respect to phenotype and function. Ontogeny studies revealed that one of these subsets ($Qa2^-$, $J11d^+$) preceded the other ($Qa2^-$, $J11d^-$) in fetal/neonatal development. This ontogeny relationship is borne out by the fact that all peripheral T cells are $Qa\text{-}2^+$, while the $Qa\text{-}2^-$ cells are found only in the thymus of the adult. We discussed previously the functional inferiority of the $Qa\text{-}2^-$ subset (20-fold) as measured by their response to plate-bound anti-T cell receptor antibodies. This difference did not appear to be due to a lack of costimulation from APC. More recent results, however, indicate that there are no differences in the ability of the two subsets to flux Ca^{++} in response to T cell receptor stimulation. In these latter responses, accessory or adherence molecules may be used to mediate or enhance receptor signaling which may be precluded in the responses to plate-bound antibodies (plus syngeneic APC). Therefore, even though the $Qa\text{-}2^-$ subset can display full functional capacity in certain responses, these cells appear to have additional requirements for T cell receptor signalling when compared to the $Qa\text{-}2^+$ thymocytes or peripheral T cells.

Previous studies from this Laboratory have demonstrated that CD4 and CD8 accessory molecules are used in the TCR-MHC interactions in both negative and positive selection. In collaboration with Richard Axel's laboratory, we have constructed CD4 and CD8 transgenic mice in order to establish the developmental stage of positive selection and the mechanism by which the accessory molecules are coordinately expressed on the appropriate MHC class-specific T cell subsets (*i.e.,* CD4 on class II-specific and CD8 on class I-specific T cells). There are two alternate models to address these questions. In the instructional model, a TCR-MHC interaction occurs at the $CD4^+8^+$ stage which co-engages the appropriate accessory molecule. This positive selection event initiates an intracellular signal to down-regulate the other accessory molecule. In the selection model, there is a random loss of one of the accessory molecules in transition from the $CD4^+8^+$ stage. Selection mediated by TCR and MHC interactions rescues only those cells expressing the appropriate accessory molecule (CD4 in class II interactions, CD8 in class I interactions). In order to distinguish between these models, we have constructed CD8 transgenic mice which constitutively express CD8.1 at high levels throughout development. In collaboration with Dr. Harold von Boehmer of the Basel Institute, we have mated these mice to $TCR\alpha\beta$

transgenics, constructed from the anti-HY (H-$2D^b$) TCR. Our results from these experiments are consistent with the instructional model, in that we do not observe the appearance of $CD4^+8^+$ cells bearing the TCR$\alpha\beta$ transgene in the double transgenics. In fact, it appears that in the double transgenic, fewer $CD4^+$ cells bearing the $\alpha\beta$ transgenes occur (presumably those selected on endogenous TCR). This result suggests that, if anything, expression of the CD8 transgene accelerates selection such that fewer endogenous receptors get expressed. This could occur if positive selection is the signal to terminate TCRα rearrangement.

One of the CD4 transgenic lines had the unusual property of only expressing the transgene on a significant proportion of the $CD8^+$ T cells in the periphery, but not in the thymus. This provided an opportunity to examine the functional consequence of CD4 expression in the population of class I-restricted $CD8^+$ cells. Functional analysis of those $CD8^+$ cells expressing the CD4 transgene revealed that these $CD4^+8^+$ cells recognize both allogeneic class I and II MHC, whereas $CD8^+$ cells from control animals react only with allogeneic class I. Since the CD4 transgene was not expressed in the thymus, it could have no affect on the selection of these cells. Therefore, these observations suggest that the ability of a T cell population to react with class II allogeneic MHC depends, to a large degree, on the presence of CD4.

(F. Ramsdell, E. Robey, and B. J. Fowlkes, LCMI, NIAID)

T Cell Memory and Tolerance

This new section in the laboratory, headed by Dr. Polly Matzinger, began on September 18, 1989. During its first year, the Section has gotten off to a roaring start and has already devised a new method to measure cytotoxic T cell activity *in vitro* and perfected a method to measure immune rejection *in vivo*. Using these new techniques in combination with a standard thymic organ culture, they have undertaken many new experiments to address the following four questions: *1)* do newly born T cells leave the thymus in a tolerizable state, or are they already functionally mature? *2)* does the thymic epithelium present antigens in a tolerogenic or immunogenic fashion? *3)* does T cell priming and/or memory require the presence of B cells? and *4)* what is the true frequency of alloreactive T cells?

Preliminary experiments suggest that newly developed $CD4^+8^-$ and $CD4^-8^+$ single-positive thymocytes are fully capable of responding to allogeneic stimulators.

(A. Bonomo and P. C. E. Matzinger, LCMI, NIAID)

Administrative, Organizational, and Other Changes

During the past year Drs. Adriana Bonomo, Kurt Brorson, and Ken Inamori joined the laboratory. Drs. Daniel Mueller and Zdenko Kovac left the laboratory.

Honors, Awards, and Scientific Recognition

Dr. Schwartz is a member of the editorial boards of *Science, International Immunology, Immunology Today, The Journal of Molecular and Cellular Immunology,* and *The International Journal of Cell Cloning.* He was also an advisory editor for *The Annual Reviews of Immunology, Vol. 10.* During the past year, he was a symposium speaker at a meeting on The Immune System and AIDS in San Moreno, Italy; a session chairman at a meeting on T Cells and Cytokines in Health and Disease held at Airlie House, Maryland; the keynote speaker at a workshop on The Role of Astrocytes in Inflammatory Demyelination at Galveston, Texas; a symposium speaker at a WHO meeting on Cellular Mechanisms in Malaria Immunity held at the NIH; a symposium speaker and session chairman at a meeting on The Molecular Mechanisms of Immunological Self-Recognition at Arden House, New York; and a lecturer at the AAI advanced course in Immunology in St. Louis, Missouri.

Dr. Daniel Mueller was a symposium speaker in the FASEB summer conference on Autoimmunity held at Saxtons River, Vermont.

Dr. B. J. Fowlkes was an invited speaker at the Third International Conference on Lymphocyte Activation and Immune Regulation in Newport Beach, California; an invited speaker at the Gordon Conference in Oxnard, California; an invited symposium chairman and speaker at the Tenth International Conference on Lymphatic Tissues and Germinal Centres in Immune Reactions in Compiégne, France; and an invited lecturer in the graduate courses at George Washington University and the Uniformed Services University of the Health Sciences Medical School. Dr. Fowlkes has also been invited to give research seminars at Duke University in Durham, N.C.; the University of Virginia Health Sciences Center in Charlottesville, VA; the Johns-Hopkins University Immunology Council, Baltimore, MD; and the State University of New York Health Sciences Center, Syracuse, NY.

Dr. P. C. E. Matzinger was an invited speaker and session chairperson at the Nordic Symposium on Fish Immunity and Infection: Evolution of the Immune System in Umea, Sweden; an invited lecturer and workshop chairperson at the VI summer school "Regulation of the Immune Response" in Stockholm, Sweden; session chairperson at a WHO meeting on Cellular Mechanisms in Malaria Immunity held at the NIH; a symposium speaker and session chairperson at a meeting on the Molecular Mechanisms of Immunological Self-Recognition at Arden House, NY; a session chairperson and invited speaker at the 10th International conference on Lymphatic Tissue and Germinal Centres in Immune Reactions in Compiegne, France; a sympsoium speaker at the European Federation of Immunological Societies, Edinburgh, Scotland; and a symposium speaker at the 4th International Club de Transplantation meeting in Les Vaux de Cernay, France. Dr. Matzinger has also been invited to give research seminars at the University of California at Berkely, CA, New York University, NY, Cornell University Veterinary School, NY, the University of California Medical School, San Francisco, CA, the Institute Pasteur, Paris, France, Hôpital Necker, Paris, France, Yale University, New Haven, CT, and the "THUG" Immunology group, London, Great Britain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00529-03-LCMI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigen Processing and Presentation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ronald H. Schwartz	Chief	LCMI, NIAID
Others:	Ephraim Fuchs	MSF	LCMI, NIAID
	Hiro Hashimoto	HHMS	LCMI, NIAID
	Calman Prussin	RA	LCMI, NIAID
	Albert Bendelac	VF	LCMI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
5.5	4.5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The antigen-presenting cell controls the outcome of T cell antigen-receptor occupancy by its ability to deliver a costimulatory signal. In the presence of costimulation the T cell makes interleukin 2 and divides. In the absence of this second signal, the T cell makes only a partial response and is induced into a nonresponsive state known as clonal anergy. Thus, different cell types bearing MHC molecules and antigen will have differential effects on the immune response depending on their ability to deliver the costimulatory signal. During the past year we have investigated the ability of small, low density ("resting") B cells to present antigen to T cells *in vivo* and *in vitro*. *In vitro* these cells stimulate a proliferative response from T cell clones, although this capacity is abolished by preirradiating the B cells with 3000R. This observation suggested that the B cells express costimulatory activity. In contrast, the same population when injected *in vivo* induced partial or complete tolerance in two systems, the cytotoxic response to the male antigen H-Y and the mixed lymphocyte response in full MHC incompatible stimulation. These observations suggested that resting B cells do not express costimulatory activity. This paradox was resolved when it was discovered that overnight incubation of resting B cells in tissue culture made them radioresistant for antigen presentation. This suggested that resting B cells lack costimulatory activity but that it is induced during *in vitro* culture. This induction process appears to be sensitive to 3000R irradiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00485-04-LCMI

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)
The Biochemical Basis of T Cell Activation and Inactivation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ronald H. Schwartz	Chief	LCMI, NIAID
Others:	Daniel Mueller (3/4)	MSF	LCMI, NIAID
	Bart Beverly	IRTA	LCMI, NIAID
	Steven Stringfellow	RA	LCMI, NIAID
	Kurt Brorson	IRTA	LCMI, NIAID
	Delanie Cassell	IRTA	LCMI, NIAID
	Ken Inamori (1/4)	VF	LCMI, NIAID

COOPERATING UNITS (if any)
Sang Kang and Mike Lenardo LI, NIAID

LAB/BRANCH
Laboratory of Cellular and Molecular Immunology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
7.5	5.5	2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of T cell activation of normal murine T cell clones of the T_H1 type have shown that two signals are required to stimulate the cells to make interleukin 2 and divide. One signal is given through the antigen-specific receptor that is uniquely expressed on each clone. The other signal is called costimulation and is delivered through a different receptor that has not yet been characterized. Signal 1 in the absence of signal 2 induces a state of hyporesponsiveness in the T cell clone known as clonal anergy. In this state, the cell fails to make significant amounts of interleukin-2 when restimulated with both signals 1 and 2. During the past year, the reasons for this failure to make IL-2 were examined at the molecular level. Although signaling through the antigen receptor was found to be normal, as assessed by increases in intracellular calcium and protein kinase C activation, steady state levels of IL-2 mRNA were decreased 20 to 50-fold. Other lymphokines were also affected but not as profoundly. Interleukin 3 production in response to stimulation was down 8-fold and gamma interferon and tumor necrosis factor production were down 3-fold.

In an attempt to extend our observations on T_H1 clones to other T cell subpopulations, we tried to induce clonal anergy in T_H2, T_H0 and freshly isolated T lymphocytes from immunized mice. Three bona fide T_H2 clones could not be anergized with any of three techniques used to anergize T_H1 clones. In contrast, two T_H0 clones could be anergized. The common theme appears to be whether the T cell clone depends on IL-2 for its autocrine growth. T_H1 and T_H0 clones, which utilize IL-2, can be anergized, whereas T_H2 cells, which utilize different growth factors, cannot. Finally, freshly isolated T cells behaved in an anomalous manner. These cells could be anergized when exposed to the calcium ionophore, ionomycin, but not when stimulated with antigen on chemically-fixed presenting cells. The reason(s) for this last paradoxical set of results is still under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00486-04-LCMI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

T Cell Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B.J. Fowlkes	Senior Investigator	LCMI,NIAID
Others:	F. Ramsdell	IRTA	LCMI,NIAID
	E. Robey	Guest Researcher	LCMI,NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

Section on Thymocyte Differentiation

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐x(c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In previous studies, we demonstrated, using radiation chimeras, that there exists a nondeletional mechanism for inducing self tolerance in the thymus. In attempts to establish the cell type responsible for this induction, it was shown that the thymic epithelium is unable to synthesize the Mls self antigen. Other experiments indicated that both $CD4^+$ and $CD8^+$ thymocytes are anergized in the chimeras for the class II-dependent antigens under study. While these data are compatible with anergy induction occurring at a $CD4^+8^+$ stage, anti CD4 treatments fail to affect the anergy displayed by $CD4^-8^+$ thymocytes. TCRαβ transgenic mice have been obtained and bred onto different MHC backgrounds. This more homogeneous systems should facilitate our future studies of thymic anergy induction.

Further work on $CD4^+8^-$ thymocytes indicates that the functional differences observed in the $Qa\text{-}2^+$ and $Qa\text{-}2^-$ subsets may be related to additional requirements for accessory and/or adherence molecules for T cell activation. Studies are in progress to establish whether these observations can be related to anergy inluction in these in thymocyte subsets.

Double transgenic mice, transgenic for CD8.1 and TCRαβ (anti-HY, H-$2D^b$-restricted) have been constructed to establish the mechanism for coordinating accessory molecule usage and MHC class specificity and to determine the stage at which positive selection occurs. The experiment was designed such that the CD8.1 transgene is constitutively expressed throughout development. The results obtained are consistent with an instructional model where the TCR-MHC interaction involves co-engagement of the appropriate accessory molecule at the $CD4^+8^+$ precursor stage. This event instructs the turn off of the other accessory molecule. The data are inconsistent with a selection model in which there is a stochastic down-regulation of one of the accessory molecules followed by TCR-MHC selection. Other studies of a CD4 transgenic line which expresses CD4 only on a proportion of peripheral $CD8^+$ T cells, and not in the thymus, provided evidence that the accessory molecules determine MHC class-specific allorecognition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00581-01-LCMI

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
T Cell Memory and T Cell Tolerance

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. Matzinger	Expert	LCMI, NIAID
Others:	A. Bonomo	VF	LCMI, NIAID

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Cellular and Molecular Immunology

SECTION
T Cell Tolerance Section

INSTITUTE AND LOCATION
NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have devised a new method to measure cytotoxic T cell activity *in vitro* and perfected a method to measure immune rejection *in vivo*. Using these techniques, in combination with our standard thymic organ culture, we are in the process of studying four questions.

1. Do newly born T cells leave the the thymus in a tolerizable or activateable state.
2. Does thymic epithelium present antigens in a tolerogenic or immunogenic fashion.
3. Does T cell priming and/or memory require the presence of B cells.
4. What is the frequency of alloreactive T cells.

Our preliminary experiments suggest that newly developed $CD4^+$ and $CD8^+$ T cells are fully capable of responding to allogeneic stimulators, thus refuting models that require the assumption that T cells leave the thymus in an anergizable state.

LABORATORY OF CLINICAL INVESTIGATION
1990 ANNUAL REPORT
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Laboratory of Clinical Investigation

Laboratory of Clinical Investigation

Summary of Report
LABORATORY OF CLINICAL INVESTIGATION
October 1, 1989 to September 30, 1990
Michael M. Frank, M.D., Chief of Laboratory
and Clinical Director, NIAID

Introduction:
This has been an extraordinarily eventful year for the Laboratory of Clinical Investigation. This year the Laboratory was reviewed by the Board of Scientific Councilors. Each program presented their progress in the past 4 years to a group of eminent scientists from outside NIAID. All of the sections were found to be strong and the research programs, in virtually all sections, were rated outstanding. We are still awaiting specifics of the report. Because of the wide variety of subjects studied by the Laboratory of Clinical Investigation only a few highlights of current studies will be listed in this summary. The section ends with a personal note by Dr. Frank, the Laboratory Chief, who announced that he will be leaving this year.

The **Clinical Immunology Section** had an outstanding year. Work is continuing on the newly described protein found in high concentration in human plasma. Because the primary function of this protein is still unknown, it is described as SGP 120 (serum sialoglycoprotein with a molecular weight of 120 kD). The protein has now been cloned and two thirds of it has been sequenced. Its unique sequence has been confirmed in multiple clones. It has been found to be present in cleaved form in the joint fluid in patients with rheumatic joint disease and partially cleaved in the joint fluid of patients with gout. Control joint fluids of patients with degenerative joint disease have shown intact protein. Earlier work using several different biological assays has shown that SGP 120 is biologically inert in its intact form. It is activated and cleaved by the plasma contact activating (kinin generating) system. In its activated cleaved form SGP 120 causes capillary leakage and smooth muscle contraction. It is not a chemotactic agent. The going hypothesis of the group is that capillary leakage and swelling as occurs in many clinical syndromes, is in part related to cleavage of SGP 120 and the generation of inflammatory peptides. Drs. Waytes and Langlois are attempting to determine whether SGP 120 is cleaved during attacks of hereditary angioedema and other types of edema. This protein was originally discovered because it acts as a complement inhibitor, binding and inhibiting the protein C4. Dr Jacobs has shown that it has complement regulatory properties.

Another major project of this group is the mechanism of action of intravenous immunoglobulin which is now used in many clinical situations. In the last year and a half the group has been studying the possibility that intravenous immunoglobulin works, in part, by acting as an acceptor for activated complement components, redirecting complement attacks away from targets. Thus, for example, cells coated with antibody may activate complement; the complement may bind to the cells and cause lysis. Although complement continues to be activated in the presence of intravenous immunoglobulin, the targets are not affected. The precise biochemical mechanism of this effect and its clinical significance are under investigation.

Ms Gaither continues to study the IgG Fc receptor and its function. It has been shown in the course of this year that IL 2 does interact with neutrophils, a previously unknown fact. It was also shown that this interaction leads to down regulation of the neutrophil activation caused by tumor necrosis factor. This finding may explain why patients with AIDS, treated with IL 2, are more susceptible to infection and thus, has important clinical implications.

The Section continues to study the effect of complement and antibody on viral lysis. Studies are being completed to determine the mechanism by which virucidal antibody causes lysis, while virus that bind complement in the absence of virucidal antibody are not lysed.

The **Mucosal Immunity Section** has also had a fine year. Probably the most important discovery of the year relates to Dr James' work on leu 8 antigen on the cell membrane of human lymphocytes. Working with Dr. Brian Seed the molecule was cloned and sequenced. It was found to be homologous to a previously sequenced molecule in the murine system named MEL 14. This molecule had been shown to be a lymphocyte homing molecule and to control the trafficking of lymphocytes. It appears that understanding the function of this molecule allows one to understand much of the homing mechanism of circulating T cells. Further studies of the chemistry of this molecule have shown that it is present in two forms on the cell surface. One form is phosphatidyl inositol linked to the membrane. On activation of cells the leu 8 antigen is shed. CD4 positive leu 8 positive cells are poor helper cells as opposed to leu 8 negative CD4 positive cells. The molecular basis for this fact is under study. These studies have helped further define subpopulations of lymphocytes that control various aspects of the immune response. This is a long-standing interest of this group.

Another of the long-standing interests of this group has been the factors that induce the switching of lymphocytes from production of IgM or IgG to that of IgA production, as occurs in the gut. The role of IL4 in expanding the population of cells capable of making IgA and augmenting the response has been defined. It has also been shown that IL5 acts as a terminal differentiation factor for B cells. What is not yet known is the initial stimulus that causes the cell to switch from making IgG to making IgA. Studies of lymphokines and the control of lymphokine secretion by cells in inflammatory bowel disease are underway. The studies make use of new technology that allows for the measurement of messenger RNA rather than for actual lymphokine protein. These studies are progressing very satisfactorily.

The **Bacterial Diseases Section** has had an enormously productive year and a half. Early work had shown the presence of 47 and 67 kD cytosolic proteins that play an important role in the respiratory burst of neutrophils and were missing in various patients with chronic granulomatous disease. In fact, it is possible to classify chronic granulomatous disease into a sex-linked form in which cytochrome b558 is usually missing and autosomal disease in which either the 47 or 67 kD cytosolic protein is missing. These proteins have now been isolated, antibodies made to them, and they have been, cloned, and sequenced. Sequence homology with other proteins has been

defined. It has been possible to show that these proteins move from cytoplasm to membrane when neutrophils are activated and monoclonal antibodies to various domains on the proteins have been identified. It has been possible to show that under some circumstances these proteins are phosphorylated and to infer that the phosphorylation is important in the function of these proteins.

Gamma interferon has proven to be highly effective in preventing infection in patients with chronic granulomatous disease even though it does not induce the presence of an oxidative burst in the phagocytic cells. The molecular basis for this fact is under study. The C5a and fMLP receptors have been expressed in Xenopus using molecular biological techniques.

The **Allergic Disease Section** has continued to make important contributions to the pathophysiologic basis of allergic disease. One of the more interesting findings of the Section this year has been the finding that cyclosporin inhibits mast cell release of mediators. This has potential important physiologic and clinical implications. Clinically, its use may help control some of our patients with poorly regulated mast cell degranulation. From the physiologic point of view it represents a new tool for the study of the events in mast cell triggering. Thus it has been possible to show that cyclosporin-treated cells do not degranulate and release mediators even though phosphoinositide hydrolysis and calcium influx into cells as well as cytosolic calcium concentrations are normal. The cyclosporin seems to affect a latter stage in the triggering process. DFP, on the other hand, inhibits PLC and inhibits phosphoinositide hydrolysis as it inhibits mast cell triggering. Thus important advances have been made in understanding the events in the degranulation process. Low molecular weight antimicrobial factors which may play a role in the prevention of sinusitis have been isolated from nasal secretions. A low molecular weight antioxidant has also been isolated from nasal secretions and has been shown to be made by the submucous glands in the nose. These glands are atrophic in patients with bronchopulmonary dysplasia who develop this severe illness. The antioxidant may be important in preventing BPD following exposure to oxidants. The oxidant has been defined as uric acid. It may be that the failure to have adequate amounts of uric acid explains some of the pathology that occurs in bronchopulmonary dysplasia.

Work continues on the isolation of a neutrophil factor that cause mast cell degranulation. One principal factor has been shown to have a molecular weight of 15,000. The factor is being isolated and sequenced. The sympathetic and parasympathetic nervous system control of the upper airway has been under investigation by this group for many years. At present, the group is focusing on neuropeptides and their importance in allergic phenomenon. Major advances have been made in identifying neuropeptide receptors in nasal and pulmonary mucosa and discovering which neuropeptides are formed that may cause capillary leakage and mucus secretion in patients with rhinitis or lung disease.

The **Mast Cell Physiology** Section has also had an excellent year. Work has continued in defining the population of patients with mastocytosis and determining

which patients require therapy and which therapies are of greatest benefit. The group now has the largest group of such patients and treatment studies are progressing. Various homing molecules present on mast cells have been identified. These belong to the recently described fibronectin, laminin leu-Cam group.

The group has also become interested in the molecular biological events that occur during mast cell growth and on mast cell triggering. Specifically, they have been looking at the formation of a series of internal proteins formed when the cells are activated. These proteins include cytokines that the cell makes in response to various signals. The approach has been a molecular biological one, isolating RNA and looking for message for the cytokines. Various patterns of message formation have been found and the interesting finding has been made that certain cytokines can be released following mast cell triggering without degranulation occurring. Thus the mast cell has the potential for regulating the function of other cells without degranulating.

The **Medical Virology Section** continues to study basic and applied clinical problems. The studies of chronic fatigue syndrome have continued. It has been shown that most patients with chronic fatigue syndrome do not have recognizable EB virus infection. Nevertheless, they have a series of psychiatric and physical disorders which are slowly being defined. For example, they do not respond normally to adrenal stimulation with appropriate release of glucocorticoids, their immunologic function has been shown to be subtlety abnormal. Thus the group is defining this patient population and attempting to determine the physiologic basis for this interesting and common disease.

Studies of herpesvirus latency have continued. Certain genes have been shown to be transcribed during the latent period and in some cases antisense transcripts are formed. The physiologic importance of these transcripts, their location in the genome, and their mechanism of action are all under study at this time. The group has been able to identify such transcripts in human trigeminal ganglia as well as sacral ganglia.
Dr. Ostrove has continued to study varicella-zoster regulatory proteins and has continued to characterize these various proteins. The approach has been to develop a library which would allow for the identification of functional homologs of HSV-1 genes. A number of these genes have been isolated and mapped. Some of these genes can autoregulate their own transcription. One possibility is that the genes code for proteins that regulate the expression of other viral genes. This would allow for orderly regulation of virus production. Molecular biological studies of VZV have also progressed. As a result of these studies it may become clear why the virus is difficult to grow and grows slowly in the laboratory. Various promoters of virally encoded genes including the deoxypyrimidine kinase gene have been identified. The promoter of this gene has been sequenced and various constructs prepared to allow the regulatory aspects of the genes to be explored. The sequential activation of various genes by the produce of individual earlier genes in VZV is now becoming clear.

Ultimately, the life cycle of the virus will be understood using this approach. The approach shown in the earlier studies, which is that of determining which genes are activated, the sequence of activation, and the controlling factors in such activation has been applied to cells doubly infected with HIV and other viral genes. The fact is that patients with HIV who acquire other viruses appear to increase their production of HIV. HSV and VZV transactivate the HIV long terminal repeat in doubly infected cells. The mechanism for this transactivation has been under study. It would appear that there are nucleotide binding proteins that are responsible for this gene regulation. These are being identified and cloned.

The **Clinical Mycology Section** under Dr. Bennett continues to study a variety of organisms, particularly cryptococci. Methods have been developed in the last year that allow for transformation of Cryptococcus neoformans. This is a major accomplishment since it has been difficult to determine which genes and which proteins are associated with virulence. Moreover, it has been difficult to introduce genes into the cryptococcus organism. New methods have been developed that allow introduction of genetic material. Models have been established in which virulence factors can be evaluated.

The group has also shown that there are two genetically distinct types of Candida albicans. The group has focused on the difference between Candida albicans and Candida stellatoidea in order to understand why the later organism is of low virulence. It has been shown that the organisms are indistinguishable in genetic terms in a number of ways. Genetic variants have been formed with defects in sugar metabolism. These differences were not responsible for differences in pathogenicity. The genetic work is continuing at this time. Beta-glucan has been shown to inhibit binding of Aspergillus fumigatus conidia to human monocytes. This appears to occur via interaction of a specific receptor on monocytes with a molecular weight of about 40- 70 kD. Work on the biochemistry and function of this receptor is proceeding.

In the last year a **Gene Therapy Unit** was established. This unit has succeeded in introducing cDNA for the 47 kd phagocyte cytosolic protein deficient in some children with CGD into murine fibroblasts and human myeloid cell lines. This new unit is interested in introducing genetic material into cells with the ultimate goal of reinfusing the cells into patients and correcting the defect in chronic granulomatous disease. The ability to clone this protein into cells in culture represents an exciting beginning into what we are sure will be an important long term goal.

At the time of the Board of Scientific Councilors' Review, Dr Frank, Chief of the Laboratory of Clinical Investigation and Clinical Director, NIAID for the past 13 years announced that he had accepted the Chair of the Department of Pediatrics at Duke University and would also be appointed Professor of Pediatrics and Medicine at that Institution. Dr. Frank has been the fourth Clinical Director in the history of the NIAID.

On a personal note, this has been an exciting and productive period. The laboratory has gone from 56 budgeted positions to 76 budgeted positions. New sections, section

chiefs, and programs have been added. New space and resources have been added as well. All tenured scientitists have been elected to the American Society of Clinical Investigation, a rigorous criterion for quality. The LCI has come to be looked upon as perhaps the leading training ground for academic clinical investigators in Allergy, Clinical Immunology and Infectious Disease. Its students have risen to positions of prominence in many of the leading medical schools in America. These have been wonderful and fulfilling years.

In passing on this leadership position I would like to thank the members of my staff who have made my success possible. In particular I would like to thank Mary Lou Eury, Thelma Gaither and Carl Hammer for their efforts on my behalf. They have helped make our Laboratory and program one of the most successful on the NIH campus. All of the senior staff, support staff, fellows, nurses, and clinical center staff have worked together in unusual harmony to produce the quality of scientific excellence in a setting of being totally committed to helping our patients with their ills. It has been a priviledge to serve with this group for these many years.

HONORS AND AWARDS

Michael Frank	EEO Special Achievement Award Deputy Editor of the JI Selected for the position of Chairman of Pediatrics at Duke
Michael Kaliner	Elected to the Council of the American Academy of Allergy and Immunology Awarded the Clemens Von Pirquet Award by Georgetown University Was invited onto the International Science Advisory Board of the Pharmacia Research Foundation
Stephen Straus	Meritorious Service Medal - PHS
Warren Strober	Deputy Editor of the JI
Martha White	Merrell Dow Scholar in Allergy Award
Mary Lou Eury	NIH Award of Merit

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00043-25 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Immunology and Chemotherapy of Systemic Mycoses

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	John Bennett, M.D., Section Head	LCI/NIAID
Other:	John Rex, M.D., Medical Staff Fellow	LCI/NIAID
	Antonia Geber, M.D., Medical Staff Fellow	LCI/NIAID
	Peter Williamson, M.D, Medical Staff Fellow	LCI/NIAID
	Virginia Kan, M.D. Guest Worker	LCI/NIAID

COOPERATING UNITS (*if any*)

Steven Schnittman, M.D., LIR/NIAID, Michael Amantea, Pharm.D., CC/NIH, Mary Cummings Smolskis, R.N.CC/NIH, Thomas McCutchan, Ph.D., LPD/NIAID

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

1. Amphotericin B lipid complex, a new formulation by E. R. Squibb Inc., was found to be well tolerated by normal volunteers but provided five fold lower blood levels than the commercial formulation and caused transient asymptomatic liver function abnormalities in 2 of 8 persons. 2. The polymerase chain reaction can detect as little Candida albicans DNA as that in a single fungal cell; the test may be useful diagnostically. 3. Gamma interferon therapy of patients with chronic granulomatous disease increased the ability of the patient's neutrophils to damage Aspgerillus hyphae, suggesting that the therapy may improve host defense against aspergillosis. 4. Polyclonal antibody was raised in rabbits against a 69 Kd human monocyte protein. This is thought to be the surface protein that facilitates host defense by binding Aspergillus spores to monocytes and macrophages.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00045-22 LCI

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Studies on Interaction of Antibody and Complement on Production of Immune Damage.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: Michael M. Frank, Chief, LCI/NIAID

Others: Carl Hammer, Senior Staff, NIAID
Tom Waytes, Medical Staff Fellow, NIAID
Paul Langlois, IRTA
Robin McKenzie, Medical Staff Fellow, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Studies of the purification and mechanism of action of a plasma protein, the C1 inhibitor, have continued. Last year we reported that we had developed a new method for preparing this difficult to purify plasma protein. We have now been studying the mechanism of action of this protein. Previous studies have suggested that this protein interacts with activated C1 to form a covalently bound complex Clr-Cls (C1 1NH) . How this complex forms is unknown. Formation of the complex is associated with disassociation of C1 and release of C1 from its target binding site. We have now shown that this result is target specific. The published result is correct when one studies antibody coated sheep red cells, the usual target examined. If one studies other targets including guinea pig red blood cells and some viruses like para influenza virus one finds that C1 inhibitor remains target bound. Analysis of this effect suggests that a complex has been formed of C1 1NH and Cls. Presumably this represents the first step in the formation of the large complex formed with sheep cells. This type of analysis will allow us to understand the various steps involved in C1 1NH mechanism of action.

Studies continue on the mechanism by which bactericidal antibody influences complement mediated lysis of targets. It appears that complement directs C4 binding to a particular viral surface protein, the HN protein, that does not bind C4 in the absence of specific anti HN antibody.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00047-21 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Studies of Patients with Known or Suspected Parasitic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. A. Ottesen	Head, Clinical Parasitology Section	LCI/LPD,NIAID
	T. E. Nash	Senior Investigator	LCI/LPD,NIAID
	F. A. Neva	Chief	LPD,NIAID
		Senior Investigator	LCI,NIAID

Others: See next page

COOPERATING UNITS (if any)

See next page

LAB/BRANCH Laboratory of Clinical Investigation

SECTION Clinical Parasitology Section

INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of these studies is to increase understanding of the pathogenesis of parasitic diseases and to improve their diagnosis, therapy and prevention. In addition to studies at NIH, clinical investigation of patients has been carried out in the endemic areas of Guatemala (onchocerciasis), India and Brazil (filariasis), Benin (loiasis), Zaire and Jamaica (strongyloidiasis), Honduras (leishmaniasis) and Panama (leishmaniasis).

Laboratory studies have permitted definition of the regulatory and pathogenetic mechanisms underlying the IgE, IgG4, and eosinophil responses to helminth infection, as well as the identification of molecules responsible for antigenic variation in giardiasis and likely involved in protective immunity in bancroftian filariasis, onchocerciasis and strongyloidiasis.

A diagnostic test to detect new and prepatent onchocerciasis infections has been developed using a purified recombinant 16 kD protein (OV-16).

Controlled clinical trials of ivermectin for bancroftian filariasis have shown it to be almost as effective as the older antifilarial drug diethylcarbamazine, and equally safe in clearing microfilaremia; because of its single-oral-dose mode of therapy, it should be considerably more practical for use in control programs than diethylcarbamazine has been.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00048-20 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Pathophysiology of Autoimmune Hemolytic Anemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Michael M. Frank, Chief, LCI/NIAID

Others: C. Hammer, Senior Investigator, LCI/NIAID
P. Langlois, IRTA
M. Basta, Visiting Fellow, LCI/NIAID

COOPERATING UNITS (if any)

L Fries, Johns Hopkins School of Public Health, Baltimore MD

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Disease, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have recently shown that the level of plasma immunoglobulin is important in controlling the degree of binding of activated complement components to targets. The immunoglobulin is believed to act as a receptor for these activated components preventing their attachment to the antibody coated targets. It appears that one major effect of intravenous immunoglobulin used for therapeutic use is to raise the level of circulating immunoglobulin. This has no effect on complement activation but diverts attack away from targets. Patients have been administered IVIG and their serum has been shown to be less effective at lysing antibody coated target cells or red cells from patients with paroxysmal nocturnal hemoglobinuria, cells that are highly complement susceptible. Patients with either autoimmune hemolytic anemia or idiopathic thrombocytopenic purpura have been treated with IVIG, known to be therapeutic. The level of red cell IgG rose since the IVIG has anti red cell antibodies. The levels of red cell C3 fell and the patients improved. Myeloma proteins have been show to vary in their ability to prevent complement attack on targets. The structural basis for the difference between proteins is under study.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00057-17 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Basic studies on pathogenic fungi

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

K.J. Kwon-Chung, Research Microbiologist LCI/CMS/NIAID
R. Kelly, Senior Staff Fellow LCI/CMS/NIAID
A. Varma, Microbiologist LCI/CMS/NIAID
B.L. Wickes, Microbiologist LCI/CMS/NIAID

COOPERATING UNITS (if any)

University of California, San Francisco

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
4.0	2.5	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Topics of current studies include: 1) Cloning of the *URA5* gene and development of a transformation system in *Cryptococcus neoformans*; 2) Characterization of stable and unstable transformants of *C. neoformans*; 3) Cloning and sequencing of a regulatory gene for α-glucosidase in *Candida albicans*; 4) Linkage mapping of *C. albicans* chromosomes.

A cDNA encoding orotidine monophosphate pyrophosphorylase (OMPP, *URA5*) of *Cryptococcus neoformans* var. *neoformans* has been isolated by complementation of *E. coli pyrE* mutants. The OMPP cDNA was then used as a probe to isolate a genomic DNA fragment containing the entire *URA5* gene. The plasmid, pURA5g2, containing the *URA5* gene was introduced into a *C. neoformans ura5* strain by electroporation.

The transformants were of three kinds: stable transformants showing homologous or ectopic integration of pURA5g2, stable transformants showing integration as well as having autonomously replicating plasmids, and unstable transformants containing only autonomously replicating plasmids.

A *Candida albicans* gene involved in sucrose utilization was cloned and the gene was found to complement the sucrose utilization defect of Type II *C. stellatoidea*. The DNA sequence showed that it encodes an open reading frame of 501 amino acids. No significant homology was found to any protein in the PRI data base. A single zinc finger motif was found at the amino terminus, suggesting that this protein has a regulatory function.

Separation of *C. albicans* chromosomes using contour clamped homogeneous electric field (CHEF) electrophoresis showed 8 bands instead of 7 as previously believed. Numerous cloned genes were used as probes to reveal the linkage group. Several genes previously thought to be located on chromosome 1 were found to hybridize to chromosome 2.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00058-16 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis and Chemotherapy of Herpesvirus Infections in Man

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: S.E. Straus	Senior Investigator	LCI, NIAID	S.Nagpal, Visiting Fellow, LCI/NIAID
J. Ostrove	Senior Staff Fellow	LCI, NIAID	
A. Freifeld	Guest Researcher	LCI, NIAID	B.Savarese, Guest Research Nurse, LCI/NIAID
D. Margolis	Medical Staff Fellow	LCI, NIAID	
D. Paar	Medical Staff Fellow	LCI, NIAID	
J. Meier	Medical Staff Fellow	LCI, NIAID	L.de Armas, Guest Worker, HHMI
P. Krause	Medical Staff Fellow	LCI, NIAID	
J. Dale	Clinical Research Nurse	LCI, NIAID	

COOPERATING UNITS (if any)

J.Rooney, Senior Staff Fellow, LOM, NIDR; A. Notkins, Chief, LOM, NIDR; J. Hilliard (Southwest Fndn. for Biomed.Res.); J. Southers, RVR; Y. Bryson (UCLA)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	4.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goals of the project are to characterize the pathogenesis, natural history and therapy of herpes simplex virus and varicella-zoster virus infections. Our clinical emphasis has been on genital herpes in normal and immune-impaired patients. Analysis of 7 years of suppressive acyclovir therapy in patients with frequently recurring herpes have shown the drug to remain effective, to be well-tolerated, and to not induce drug resistance. We continue to study the ability of ultraviolet light and other physical and chemical agents to induce recurrent herpes simplex infections in humans. In a placebo-controlled trial, acyclovir was found to significantly block u-v induced reactivation of HSV-2 infection. A placebo-controlled trial of acyclovir for suppression of frequent, spontaneously recurring oral herpes is near completion.

The major thrust of our laboratory effort in this project has been the analysis of HSV latency in human ganglia. We demonstrated and further characterized latent HSV-2 RNAs in sacral ganglia during the past year. Further efforts to define the structure, kinetics, and regulation of these latency-associated RNAs and their promoters are underway.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00154-15 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immediate Events in Hypersensitivity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
Others:	Martha V. White, M.D.	Senior Staff Fellow	LCI/NIAID
	Mary E. Revenis, M.D.	Special Volunteer	LCI/NIAID
	Yasushi Igarashi, M.D.	Fogarty Visiting Fellow	LCI/NIAID

COOPERATING UNITS (if any)

Julie Goff, Ph.D., Georgetown University (Contract # NO-1-AI-22665)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	2.3	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This broad-based project explores allergic mechanisms at a number of levels. Antidromic nerve stimulation is an excellent model for exploring the role that endogenous neuropeptides play in disease. We have characterized neurogenic inflammation in rat cutaneous and pulmonary models and have searched for a possible connection to mast cell activation. Our studies indicate that mast cells are not activated by endogenous neuropeptide release and that neuropeptide release does not play a major role in allergic responses. We have contrasted neutrophil derived histamine releasing factor with a number of other mast cell activators, and have found HRA-N to be unique in regards to the spectrum of its activity, the kinetics of mast cell activation, and its biologic characterization. Bronchopulmonary dysplasia can be discriminated from hyaline membrane disease by the relative absence of lactoferrin and lysozyme in tracheal aspirates. This finding suggests that the relative absence of serous cell products predisposes to bronchopulmonary dysplasia (BPD). Bronchopulmonary dysplasia affects 40% of children weighing less than 2 kg. It is estimated that each child with BPD costs society $300,000/year for hospital-related expenses. Our finding of reduced serous cell products in this disease provides the first insight into a pathologic process which may be involved. Moreover, these findings provide a new insight into possible therapeutic interventions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00155-15 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Phagocytic Cell Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John I. Gallin, M.D.	Scientific Director, DIR,	NIAID
Others:	Harry L. Malech, M.D.	Head, Bacterial Dis. Sect.	LCI/NIAID
	Daniel Rotrosen, M.D.	Senior Staff Fellow	LCI/NIAID
	Philip Murphy, M.D.	Medical Staff Fellow	LCI/NIAID
	Thomas Leto, Ph.D.	Senior Staff Fellow	LCI/NIAID
	Stuart Abramson, M.D.,Ph.D.	Med. Staff Fellow	LCI/NIAID
	Richard Kenney, M.D.	Medical Staff Fellow	LCI/NIAID
	Ellen DeCarlo, R.N.	Research Nurse	DIR/NIAID

COOPERATING UNITS *(if any)*

D Kuhns, PRI-Frederick Cancer Res. Facil.; K Lomax, W Strober, M Kaliner, J Rex, and J Bennett, LCI/NIAID; W Nauseef, and R Clark, Univ. of Iowa; A Segal, University College, London, UK.

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Bacterial Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.1	2.5	0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to determine the genetic basis of inherited diseases affecting neutrophil function and associated with recurrent infections, and diagnosis and treatment of such disorders. Chronic Granulomatous Diseases of Childhood (CGD) are a group of diseases with common phenotype including absent H2O2 production by phagocytes, recurrent infections, and granuloma formation. We have documented the prevalence of the four distinct genetic forms of CGD (60% X-linked defect in cytochrome b558 gp91-phox subunit; 5% autosomal recessive (AR) defect in cytochrome p22-phox subunit; 30% AR affecting p47-phox cytosolic oxidase factor; 5% AR affecting p67-phox cytosolic factor). Studies of p67-phox gene structure reveals two normal allelic HinDIII restriction enzyme patterns which will be useful in prenatal diagnosis and mapping chromosome 1q25 region. In other studies expression of p47-phox by retroviral vector provides an important tool for eventual genetic reconstitution of this form of CGD. We have documented the effectiveness of low dose steroids in managment of obstructive granulomas of gastrointestinal and urinary system. We have documented the importance of early surgical extirpation of pulmonary aspergillus infections and drainage of liver abscesses. Review of our CGD patients' histories indicates that prophylactic trimethaprim-sulfa reduces bacterial infections without causing an increase in fungal infection. Using an in vitro assay of fungal killing by neutrophils, we have documented that a small number of normal neutrophils act synergistically with a larger number of CGD neutrophils to kill aspergillus hyphae providing a rationale for use of granulocyte transfusions in infected CGD patients.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00249-09 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Pathogenesis, Diagnosis, and Treatment of Systemic Mast Cell Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Dean D. Metcalfe, M.D.	Head, Mast Cell Physiology Section	LCI/NIAID
Others:	JoAnn Mican, M.D.	Medical Staff Fellow	LCI/NIAID
	John Costa, M.D.	Medical Staff Fellow	LCI/NIAID

COOPERATING UNITS (if any)

Clinical Pathology Department, NIH Clinical Center (Dr. William Travis)
CHB, NHLBI (Dr. Neal Young)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.00	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Fifteen adults with mastocytosis were entered into a double-blind clinical protocol comparing azelastine, an H1 antihistamine, with mast cell stabilizing properties, with chlorpheniramine. Agents were compared with symptom scores, by analyzing plasma and urine histamine levels, and by comparing suppression of skin test responses to degranulating agents. Initial data analysis suggests that azelastine was superior to chlorpheniramine at suppressing skin test responses and in reducing symptom scores of skin irritability. Azelastine, however, did not lower plasma or urine histamine levels or significantly affect other parameters of disease.

Lesional and non-lesional skin of subjects with mastocytosis was analyzed for the distribution and concentration of tryptase positive, chymase negative mast cells (MC_T) and tryptase positive, chymase positive mast cells (MC_{TC}) and compared to normal skin and non-lesional skin of subjects with unexplained anaphylaxis or flushing episodes. MC_{TC} cells were the only type of mast cells seen in all specimens analyzed. The concentration of mast cells in the superficial dermis of mastocytosis lesions (40985$\pm$21772 mast cells/mm^3) was significantly increased over that in corresponding areas of non-lesional skin from subjects with mastocytosis (7178$\pm$3607 mast cells/mm^3), skin from subjects with idiopathic anaphylaxis or flushing episodes (6974$\pm$3873 mast cells/mm^3) and normal skin (7347$\pm$2973 mast cells/mm^3). The exclusive presence of MC_{TC} cells in skin lesions of mastocytosis which are characterized by non-malignant hyperplasia of mast cells suggests involvement of local tissue factors in mast cell recruitment and differentiation.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00271-09 LCI

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Purification and Characterization of Complement Proteins and Fragments

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Carl H. Hammer, Senior Investigator, LCI/NIAID
Others: Michael M. Frank, Chief, LCI/NIAID
Thomas A. Russo, Senior Staff Fellow, LCI/NIAID
Thomas L. Leto, Senior Staff Fellow, LCI (Bacterial Diseases Sec.)/NIAID
Ruth M. Jacobs, Medical Staff Fellow, LCI/NIAID
Paul Langlois, IRTA, LCI/NIAID
Yannick Pilatte, Visiting Fellow, LCI/NIAID
Gilda Linton, Medical Technologist, LCI/NIAID

COOPERATING UNITS *(if any)* Lois Renfer, Chemist, LCI/NIAID

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
National Institutes of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.9	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Sgp120 is a new plasma sialoglycoprotein of 120 kDa on which we have recently reported the isolation and partial characterization. This protein was co-isolated with the second component of human complement (C2) on C4b-Sepharose. While these proteins have features in common, the following important differences have been noted. Sgp120 is present in plasma at 15x the concentration of C2. These proteins do not cross immunoprecipitate with polyclonal antisera. They are activated and cleaved by distinct mediator pathways: sgp120 by the contact-activation system (CAS) and C2 by the classical complement pathway (CCP). The proteins have unique N-terminal amino acid sequences and a C-terminal stretch of 238 residues within sgp120 as determined by cDNA cloning and sequencing is unrecognized in any of 55,000 screened protein sequences including C2, kallikrein and HMW kininogen. Affinity isolated sgp120 (sgp120-A) represents a small % of the total available sgp120 in plasma. Sgp120 (sgp120-I) which doesn't bind to C4b can by isolated by conventional chromatography also contains a small % of sgp120-A. Although the two forms are immunochemically indistinguishable, produce similar fragments on kallikrein digestion and have identical N-terminal amino-acid sequences, by a number of criteria these two forms are distinct and in particular sgp120-A possess most if not all described biologic activities. Sgp120-A is slightly larger in size and has a more basic pI of 5.0. We have fully sequenced the initially identified and isolated clone that produces a fusion protein detectable by monospecific antibody to sgp120. The partial deduced 238 aa sequence contained the N-terminal peptide sequence derived from a C-terminal 25 kDa fragment derived from sgp120-A. We are in the process of completing the sequence of larger clones identified by ^{32}P labeled probe hybridization with cDNA from our initial clone.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00275-09 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Complement Receptor and C3-Mediated Opsonization

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Thelma A. Gaither, Senior Investigator

Others: Michael M. Frank, Chief, LCI/NIAID
Marva Moxey-Mims, Guest Researcher
Hank Simms, Guest Researcher
Evelyn Lin, Chemist (technician)
Tiffani Durant, Laboratory Worker

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institutes of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.75	2.0	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several cytokines that affect granulocyte functions increase in inflammatory conditions, and it is known that patients on IL-2 therapy have an increased risk of infection. Our objective was to explore the effects of three cytokines, IL-1, IL-2 and TNF, on PMN phagocytosis, with particular reference to the possible effect of IL-2 on TNF and IL-1, and the role they may play in the upregulation of Fc R activity in acute bacterial infections, which we had previously reported. Sheep RBC opsonized with IgG were used as targets and phagocytosis by adherent PMN was observed microscopically. Dose response studies showed that phagocytosis was markedly increased by IL-1 and TNF . The combination of IL-1 and TNF, at optimal concentrations, had an additive effect. IL-2 alone did not affect phagocytosis ; however, the upregulatory effect noted with TNF, as well as with the combination of TNF and IL-1, was reduced by IL-2. IL-2 did not inhibit Il-1 activity.The cytokines did not alter expression of PMN FcR's; there was no increase in binding of ^{125}I-MAbs against FcR I, FcRII or FcRIII. It was shown that IL-2 inhibited the TNF effect by blocking the binding of TNF to PMN. A MAb against the low-affinity IL-2 receptor (anti-Tac), reversed the inhibitory effect of IL-2 on TNF, wheras a MAb against the intermediate-affinity IL-2 receptor (mikß1) had no effect. Notably, IL-2 down-regulated TNF only with adherent PMN ; it did not affect ^{125}I-TNF binding to PMN nor the TNF upregulation of phagocytosis by PMN that were in suspension. This suggested that a surface-expressed IL-2 receptor on adherent PMN may be involved in IL-2 down-regulation of TNF activity. In agreement with others, we did not detect IL-2 receptors on PMN in suspension by FACS using anti-Tac and mikß1; however, both MAb's stained permeabilized PMN by indirect immunofluorescence, and from 500 to 1000 binding sites /adherent PMN were detected using 125I-anti Tac MAb.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00278-09 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Components of the Complement Cascade

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Carl H. Hammer, Senior Investigator, LCI/NIAID
Others: Michael M. Frank, Chief, LCI/NIAID
Ruth M. Jacobs, Medical Staff Fellow, LCI/NIAID
Paul F. Langlois, IRTA, LCI/NIAID
Gail S. Kerr, Visiting Fellow, LCI/NIAID
Yannick Pilatte, Visiting Fellow, LCI/NIAID
Gilda Linton, Medical Technologist, LCI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institutes of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.75	.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have recently identified a novel sialoglycoprotein present in human blood and body fluids. This protein has a molecular mass of 120 kDa (sgp120) and has a single chain structure. Like the second component of complement (C2), this protein binds to activated C4b of the classical complement pathway (CCP) and can be recovered from Sepharose linked C4b as part of an isolation and purification scheme. The sgp120-A isolated by this technique has been shown to regulate the assembly of the CCP C3 and C5 convertases as demonstrated by hemolytic site inhibition in a specific and dose-dependent fashion. Use of radiolabeled sgp120-A showed its specific binding to C4b bound to antibody, sensitized sheep erythrocytes (EAC4b). Binding was both dose responsive and saturable showing specificity for C4b. Sgp120-A binding was inhibitable by both cold sgp120 and C2. Preliminary data suggest that sgp120-I, the predominant form of the protein which does not bind to Sepharose bound C4b, binds minimally to EAC4b and that much larger amounts of sgp120-I are required for inhibition of the CCP, if inhibition occurs at all. As earlier reported, sgp120 fragments possess both vasodialation and contractile capacity as demonstrated in separate guinea pig models. Sgp120 fragmentation was assessed in the plasma of individuals with the swelling disorder hereditary angioedema (HAE). No associated fragmentation of sgp120 was evidenced in individuals experiencing severe, active swelling. However, sgp120 was cleaved into the characteristic fragments in all persons with inflammatory joint disease, but not in individuals with noninflammatory, joint disease.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00279-09 LCI

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Studies on Mucous Glycoproteins

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator*) (*Name, title, laboratory, and institute affiliation*)

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
Others:	Joaquim Mullol, M.D.	Special Volunteer	LCI/NIAID

COOPERATING UNITS (*if any*)

James H. Shelhamer, M.D., Carolea Logun, and Jens D. Lundgren, M.D., Critical Care Medicine, Clinical Center, NIH

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Allergic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Mucus secretion is a normal function of respiratory mucous membranes. Models for measurement of mucus production by cultured human and feline bronchial and nasal mucosae have been developed in order to examine the controls of mucus secretion. Using feline airway, PAF and GRP caused mucus secretions, and the isolated eosinophil granule protein, ECP, was even more active. Thus, we are continuing to analyze factors possibly participating in asthma for their actions on mucus secretion.

We also compared our human in vivo challenge system with in vitro responses and confirmed that both models are necessary to explain nasal secretory controls.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00354 08 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Immunoregulatory defects in inflammatory bowel disease

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

Stephen P. James, M.D., Investigator, Mucosal Immunity Section, LCI, NIAID

Yohko Murakawa, M.D., Visiting Associate, Mucosal Immunity Section, LCI, NIAID

COOPERATING UNITS (*if any*)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The inflammatory bowel diseases (IBD) Crohn's disease and ulcerative colitis are debilitating diseases of unknown etiology that affect 1-2 million individuals in the U.S. The current objective of this project is to further define the role that mucosal T cells play in the pathogenesis of IBD. The specific focus is to determine the mediators released by T cells in lesions, and to determine the T cell receptor specificity of lymphocytes in lesions. Previous studies of lymphokine production by IBD T cells have depended on *in vitro* activation of lymphocytes isolated from enzymatic digests of intestinal tissues, and there is no information regarding lymphokine production by T cells *in situ*. In current studies RNA was isolated from colonoscopic biopsies from patients with inflammatory bowel disease and noninflammatory controls (colon cancer or suspected cancer). We developed a sensitive method for quantitation of lymphokine mRNA expression in endoscopic biopsies. RNA was extracted from intestinal biopsies, reverse transcribed into cDNA, and amplified by PCR for IL-2. Dilutions of synthetic IL-2 mRNA transcripts that were slightly longer than native IL-2 were added to RNA samples as internal standards. IL-2 mRNA was detectable in as few as 100 activated lymphocytes or about 1 pg of mRNA, and dose response curves showed that there was a quantitative relationship between input mRNA and PCR products over a multiple log range. Using this method, we have found that expression of IL-2 was increased in biopsies of IBD patients who have active inflammation. This is the first demonstration of an abnormal increase in lymphokine expression in IBD. The proposed course will take advantage of this technology to define the T cell mediators expressed in inflammatory lesions and identify the T cell receptors expressed by T cells in IBD lesions. These results may serve as the basis for novel immunotherapies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00355-06 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunoregulatory defects in primary biliary cirrhosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Stephen P. James, M.D., Mucosal ;Immunity Section, LCI, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was not active during this period.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00356-08 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Regulation of IgA Immunoglobulin Synthesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. Warren Strober, M.D., Head, Mucosal Immunity Section, LCI/NIAID
Other Gregory R. Harriman, M.D., Medical Staff Fellow, LCI/NIAID
Kirsi C. Allison, Howard Hughes Medical Fellow, LCI/NIAID
Ker Sang Chen, Ph.D., Senior Staff Fellow, DV/FDA

COOPERATING UNITS (if any)

Division of Virology, FDA

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During this period we conducted a number of studies of the role of IL-5 and other lymphokines in B cell differentiation, particularly IgA B cell differentiation. In one set of studies we performed an extensive evaluation of IL-5 receptor (IL-5R) expression on B cell lines and on stimulated normal B cells. We found that IL-5 binds to a high affinity and low affinity receptor on the B cell surface. Stimulation of normal B cells with α-δ-dextran, greatly augments high affinity IL-5R expression, whereas α-μ antibody or PMA/Ionomycin causes only minimal increases in IL-5R expression. In addition, LPS does not increase IL-5R expression and inhibits that induced by α-δ-dextran. These results indicate that IL-5R expression on B cells requires activation of the latter with agents that extensively cross-link surface Ig. In addition, they suggest that certain bacterial surface components may act to down-regulate immune responses by inhibiting IL-5R expression.

In other studies, we continued to explore the effect of IL-5 and other lymphokines of B cell differentiation. We induced antigen-specific B cell responses in Peyer's patches by oral administration of mice with inactivated influenza virus and cholera toxin adjuvant. We then determined the capacity of the induced IgA B cells to undergo terminal differentiation *in vivo*, in the presence of antigen and various lymphokines. We found that IL-5 and IFN-γ could both act as independent terminal differentiation factors for IgA B cells and that these substances together gave additive effects. The highest IgA responses were obtained, however, with IL-4, IL-5 and IFN-γ. These results indicate that combinations of lymphokines are necessary to achieve maximal IgA B cell differentiation.

Finally, we continued studies of the mechanism of action of cholera toxin on B cell responses. In these studies we determined the effect of cholera toxin (CT) on purified B cells stimulated either with LPS-alone or LPS plus IL-4. We found that CT induces preferential differentiation of IgG1 B cells and augments IL-4 induced IgG1 B cell differentiation. In addition, we found that CT induced γ1 germline mRNA transcripts and augments γ1 germline mRNA transcripts induced by IL-4. These studies indicate that CT acts to induce IgG1 at the level of isotype switching.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00357-05 LCI

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Studies of the Autologous Mixed Lymphocyte Reaction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I. Warren Strober, M.D., Head, Mucosal Immunity Section, LCI/NIAID
Other Stephen P. James, M.D., Senior Investigator, Mucosal Immunity Section LCI/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Mucosal Immunity Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been inactive during the current period.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00397-07 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interactions of C3b with Immunoglobulin G-Regulation of C3b Function by Antibody

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Michael M. Frank, Chief, LCI/NIAID

Others: Milan Basta, Visiting Fellow, LCI/NIAID
Paul Langlois, IRTA/LCI

COOPERATING UNITS (if any)

Louis F. Fries, Johns Hopkins School of Public Health

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues xx (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

All of our laboratory projects have interrelated themes. The work of this project has come to overlap Z01 AI 00397-07 LCI. We have found that intravenous immunoglobulin, used to treat that syndrome, decreases cellular C3 and increased erythrocyte IgG. The latter is true because of anti RBC IgG in the IVIG preparation. See the related report for details.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00429-06 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Nasal Responses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
Others:	Gordon D. Raphael, M.D.	Medical Staff Fellow	LCI/NIAID
	Aleksander Z. Gawin, M.D.	Special Volunteer	LCI/NIAID
	James N. Baraniuk, M.D.	Special Volunteer	LCI/NIAID
	David B. Peden, M.D.	Medical Staff Fellow	LCI/NIAID
	Michiko Okayama, M.D.	Special Volunteer	LCI/NIAID
	Elizabeth V.M. Jeney, M.D.	Special Volunteer	LCI/NIAID

COOPERATING UNITS (if any)

James H. Shelhamer, M.D., Critical Care Medicine, Clinical Center, NIH

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Allergic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐x (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The nasal mucosa is the first internal surface to encounter aeroallergens, airborne pathogens, and airborne toxins. Analysis of nasal responses will likely provide insights into normal host defense mechanisms. Provocation of human and guinea pig nasal mucosa stimulates secretion, and analysis of the components of these secretions has revealed new insights into the source of these secretions as well as possible mechanisms for the pharmacologic control of secretion. Allergen challenge results in a direct vascular permeability due to mast cell derived mediators and reflex glandular secretions. An anti-inflammatory agent, nedocromil sodium, had no protective effect on responses caused by allergen. The three divisions of innervation in the nasal mucosa were found to have discrete associations with neuropeptides: the sensory nerves contain GRP (a glandular stimulant), CGRP, SP, and NKA (which act to regulate vasodilation and vascular permeability); the parasympathetic nerves contain VIP (a potent glandular stimulant); and sympathetic nerves contain NPY (a vasoconstrictor). Bradykinin, which may be generated during URIs, was found to stimulate the vascular bed but not the glands directly; thus, bradykinin may participate in the vascular permeability seen in colds. Patients with recurrent sinusitis were found to have a singular defect in nasal secretory responses to cholinergic stimulation. Therefore, these patients may be predisposed to recurrent infections by the absence of specific and nonspecific host defense molecules. Uric acid was found to be the major, stable antioxidant in secretions. Uric acid secretion appeared to be glandular in origin.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00430-06 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Molecular Biology of Varicella Zoster Virus Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S.E. Straus	Senior Investigator	LCI,NIAID
	J. Ostrove	Senior Staff Fellow	LCI,NIAID
	P. Krause	Medical Staff Fellow	LCI,NIAID
	S. Nagpal	Visiting Fellow	LCI,NIAID
	D. Paar	Medical Staff Fellow	LCI,NIAID
	D. Margolis	Medical Staff Fellow	LCI,NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project constitutes one of the two major laboratory research focuses of this section. The goals are to identify, map and characterize VZV genes and proteins expressed during active and latent infections, and to define the temporal sequence and control of gene expression. To date, we mapped the viral genome, its transcripts, and a number of important genes. We proved that VZV remains latent in satellite cells of human nerve ganglia and that 4 to 11 of 72 VZV genes are expressed during latency. To better detect and characterize latency transcripts, we are currently extracting RNA from human ganglia, processing it with reverse transcriptase, and performing polymerase chain reactions (PCR) using synthetic olio nucleotides representing targeted VZV open reading frames. In other studies, we are defining the roles and components of open reading frames responsible for VZV gene regulation. Specifically, by creating mutations within genes 4 and 61, we identified protein domains responsible for gene transactivation and suppression.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00 432 06 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Regulation of Immune responses in nonhuman primates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Stephen P. James, M.D., investigator, Mucosal Immunity Section, LCI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is aimed at determining the molecular basis for CD4 T cell regulatory functions in the intestinal mucosa. We have prevously shown that the majority to T cells in the intestinal lamina propria have the CD4+, Leu-8 - phenotype and that the Leu-8 molecule is the human lymph node homing receptor. A particular focus during the present period has been to determine the mechanisms by which CD4 T cell subpopulations mediate helper activity vs. inhibitory activity for B cell differentiation. Activated CD4+, Leu-8+ T cells had substantially lower levels of mRNA for IL-2, IL-4, IL-5 and IFN-gamma than CD4+, Leu-8– T cells. However, mRNA levels for actin, c-myc IL-2R and TGF-beta were similar in both subsets. Addition of IL-1, IL-2, IL-5, IL-6 or low molecular weight BCGF to pokeweed mitogen (PWM)-stimulated cultures did not reverse the low helper activity of CD4+, Leu-8+ T cells. Most importantly, in PWM-stimulated cultures containing B cells and CD4+, Leu-8– helper T cells, CD4+, Leu-8+ T cells suppressed Ig production when they were in direct contact with B cells. In contrast, there was little suppression when CD4+, Leu-8+ T cells were separated from B cells by a millipore membrane. Thus, the low helper activity of CD4+, Leu-8+ T cells may be due to an inherently lower capacity to produce B cell differentiation factors; their suppressor activity requires T-B contact and may be due to the release of novel inhibitory factors or a cytolytic mechanism. Future studies will determine the role of the Leu 8 molecule in contact dependent lymphocyte function and the mechanism of suppressor function of CD4+, Leu-8+ T cells.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00469-05 LCI

PERIOD COVERED

July 1989 - July 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Complement: Studies in Viral Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Robin McKenzie, M.D., LCI, NIAID
Michael Frank, M.D., LCI, NIAID

COOPERATING UNITS (if any)

Brian Murphy, M.D., LID, NIAID

LAB/BRANCH

LCI

SECTION

Clinical Immunology

INSTITUTE AND LOCATION

NIAID/NIH

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of the present study is to examine the role of a monoclonal antibody in directing complement-mediated neutralization of parainfluenza virus type 3. This model may provide information about antibody interaction which will be applicable to vaccine development and other areas of research. Even though neither complement nor the monoclonal antibody alone produce significant viral neutralization, together they neutralize the virus. This effect does not appear to be due simply to an increase in the amount of complement bound. Likewise, there is no significant increase in the percentage of C4 that is complexed to C3. Preliminary results suggest that this antibody may direct deposition of C4 to a region of the HN protein which is crucial for lysis of the virus.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 A1 00470-05 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chronic Epstein Barr Virus Infection and Chronic Fatigue Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S.E. Straus	Senior Investigator	LCI,NIAID
OTHER:	J. Dale	Clinical Research Nurse	LCI,NIAID
	B. Savarese	Guest Research Nurse	LCI,NIAID

COOPERATING UNITS (if any)

M. Kruesi (LCS), J. Grafman (LMN, NINCDS), M. Dalakas (NINCDS), M. Demitrack, P. Gold (NIMH); W. Strober, M. Sneller (LCI, NIAID); Scott Fritz (PRI, FCRF)

LAB/BRANCH
Laboratory of Clinical Investigations

SECTION
Medical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 02892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goals of this project are to characterize severe chronic infections with Epstein Barr Virus and to characterize multiple aspects of the chronic fatigue syndrome. To date this research project has involved nearly 150 patients. Included are 7 patients who were diagnosed with severe chronic EBV infections on the basis of clinical, historical, molecular and serologic features.

Detailed immunologic, neurologic, endocrinologic, and psychologic studies are being conducted on selected patients with chronic fatigue. To date we have found no consistent abnormality in the chronic fatigue patients although some can be shown to have immune abnormalities, or depression. We continue open therapeutic trials of tricyclic or MAO inhibitor compounds. During the past year we completed an initial set of studies of the pituitary adrenal responsiveness to corticotropin releasing hormone and to ACTH. We found statistically significant reductions in cortisol levels and ACTH responsiveness to CRH. These led us to propose that there is a novel neuroendocrine effect that may indicate deficient central CRH release. Since CRH induces CNS arousal, these neuroendocrine findings suggest a new mechanism whereby the lethargy of Chronic Fatigue Syndrome patients may be explained.

During the past year we explored prolonged intravenous acyclovir and recombinant gamma interferon as treatments for selected patients with life threatening complications of chronic EBV infection. The results are mixed.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00481-05 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Receptors and Transduction Mechanisms in Human Phagocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Harry L. Malech, M.D.	Head, Bacterial Dis. Sect.	LCI/NIAID
Others:	John I. Gallin, M.D.	Scientific Director, DIR,	NIAID
	Daniel Rotrosen, M.D.	Senior Staff Fellow	LCI/NIAID
	Philip Murphy, M.D.	Medical Staff Fellow	LCI/NIAID
	Thomas L. Leto, Ph.D	Senior Staff Fellow	LCI/NIAID
	Cheung Kwong, M.D., Ph.D.,	Medical Staff Fellow	LCI/NIAID
	Rachel Levy, Ph.D.,	Visiting Associate	LCI/NIAID
	Karen J. Lomax, M.D.	Senior Staff Fellow	LCI/NIAID

COOPERATING UNITS (if any) Elaine Gallin, Dept. Physiol., Arm. Forc. Rad. Biol. Inst.; Julie Katkin and Michael Kleinberg, Special Volunteers, LCI/NIAID; William Nauseef and Robert Clark, University of Iowa.

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Bacterial Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.8	3.4	1.4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project defines the biochemical structure and function of receptors, transduction proteins and the NADPH oxidase effector system involved in human neutrophil and monocyte responses to inflammation and infection. Studies focus upon: (i) chemotactic receptors for formyl peptides (FPCR), C5a, platelet activating factor (PAF) and ATP (P2); and (ii) NADPH oxidase components in the cytosol p47-phox, p67-phox, NCF-3 and the membrane cytochrome b_{558} (CYTO b) subunits p22-phox and gp91-phox mediating microbicidal superoxide production. Using myeloid cell mRNA in a frog oocyte expression system we demonstrate chloride currents and calcium effluxes in response to FMLP, C5a, PAF, and ATP. These responses are mediated by mRNA transcripts of 2 kb for FMLP and C5a receptors and 4 and 6 kb for PAF and ATP. FMLP, C5a and ATP responses are pertussis toxin sensitive (mediated by G-protein) while PAF responses are not. We are the first to delineate and clone cDNA encoding two neutrophil cytosol factors p47-phox and p67-phox essential to NADPH oxidase activation. Both proteins contain a pair of homologous sequences with similarity to the src-oncogene SH3 (A-box) regulatory region. We show that p47-phox undergoes a series of phosphorylations culminating in the interaction of p47 with a distinct amino acid sequence (RGVHFIF) present in the cytoplasmic domain of cytochrome b558 gp91 subunit. This is followed by translocation of other cytoplamic oxidase components to the membrane and activation of the oxidase. Using a baculovirus expression system, active recombinant p47-phox and p67-phox have been used to demonstrate the absolute requirement for a third cytoplamic factor, NCF-3, for activation of the oxidase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00495-04 LCI

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biochemical Events in Mast Cell Secretion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
Others:	Robert J. Hohman, Ph.D.	Expert	LCI/NIAID
	Thomas Hultsch, M.D.	Special Volunteer	LCI/NIAID
	Prescott Atkinson, M.D.	Medical Staff Fellow	LCI/NIAID
	Raynaldo A. Martin	Biologist	LCI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Allergic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.85	1.1	1.75

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐x (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The effects of cyclosporin A (CSA) on IgE receptor-mediated exocytosis from rat basophilic leukemia (RBL) cells was examined. Both IgE receptor-mediated and Ca^{2+} ionophore-induced degranulation were inhibited 50% in the presence of 0.2 µg/ml CSA. This is the concentration of CSA achieved in the plasma of patients undergoing immunosuppressive therapy with CSA. Whereas inhibition of lymphokine expression requires lengthy (hours) preincubation with the drug, maximum inhibition of degranulation from RBL cells occurs after a 5 min preincubation with CSA. CSA does not have to be present in the buffer at the time of stimulation. If RBL cells are incubated with CSA for 15 min, washed, and triggered in the absence of CSA, release is still inhibited. The inhibition of degranulation is not limited to RBL cells. IgE receptor-mediated histamine release from human basophils was inhibited by the same range of CSA concentrations that inhibits release from RBL cells.

Concentrations of CSA that results in maximum inhibition of secretion have no effect on "early" events in signal transduction such as receptor-mediated PI hydrolysis, Ca^{2+} influx, or the rise in the concentration of Ca^{2+} in the cytosol. Moreover, actin polymerization and cell viability are not affected. In addition, not all secretory cells are inhibited by CSA. Degranulation of rat pancreatic acinar cells in response to bombesin is not inhibited by concentrations of CSA that causes maximum inhibition of release from RBL cells and human basophils. These results demonstrate that the early events in signal transduction are not affected, and suggest that the intracellular target for CSA participates in a later stage of exocytosis. Furthermore, the data suggest that CSA suppresses other cells than T-lymphocytes and predict that patients on CSA therapy may have altered response to allergens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00496-04 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interactions Between the Human Immunodeficiency Virus (HIV) and Herpesviruses.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:

J.M. Ostrove	Senior Staff Fellow, LCI, NIAID
S. Nagpal	Visiting Fellow, LCI, NIAID
D. Margolis	Medical Staff Fellow, LCI, NIAID
A. Rabson	Expert, LMM, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To define the exact elements on the HIV LTR that are responsible for transactivation by HSV, we studied the effect of HSV-1 infection, or transfection with HSV-1 ICPO or ICP4, on the activation of HIV LTRs which contain mutations that delete either the SP1 binding sites, the NF-kB sites, or both NF-kB and SP1 binding sites (double mutants). Lesions in either the SP1 or NF-kB sites are not lethal to the virus.Virus with the double mutant, however, does not replicate. We studied the effect of HSV-1 infection and its immediate early genes on the activation of these HIV LTR CAT constructs in both Vero and Jurkat cells. In Vero cells, HSV-1 infection, and ICP4 or ICP0 alone can activate either of the single mutants, with the SP1 deleted mutant being activated less that the wild type. The double mutant could not be activated. In Jurkat cells similar results were obtained with each HSV-1 transactivator. Surprisingly, if cells were treated with PMA, PHA, and superinfected with HSV-1, we were able to activate the expression of the double mutant. This finding led us to postulate that an additional target on the HSV LTR may respond to HSV-1 infection.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00513-03 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of Mast Cell Growth and Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Dean D. Metcalfe, M.D.	Head, Mast Cell Physiology Section	LCI/NIAID
Others:	Helen Thompson, Ph.D.	Visiting Scientist	LCI/NIAID
	John Costa, M.D.	Medical Staff Fellow	LCI/NIAID
	JoAnn Mican, M.D.	Medical Staff Fellow	LCI/NIAID
	Naveen Arora, M.D.	Visiting Fellow	LCI/NIAID

COOPERATING UNITS (if any)

NIDR (Y. Yamada)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.25	3.75	0.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Both human basophils and mast cells arise from CD34+ progenitor cells. Messenger RNA's for the alpha, beta, and gamma subunits of the high affinity IgE receptor receptor are coordinately expressed during the first few days of culture of murine bone marrow coincident with the appearance of mononuclear cells bearing high affinity IgE receptors. IgE receptor positive cells purified by FACS have been demonstrated to develop into mature mast cells. Mast cells adhere to surfaces coated with laminin. This process is enhanced by IgE-mediated activation but may occur in the absence of histamine release. Mast cell attachment to laminin depends upon a five amino acid (IKVAV) sequence. When mast cells attach to laminin, they decrease c-myc, but increase c-fos expression; and the levels of nuclear factors for c-AMP responsive elements increase. Murine mast cells also adhere to fibronectin, a process which is also dependent upon mast cell activation. Adhesion is inhibited by the RGDS peptide. A 120 kD fragment of fibronectin containing an attachment region mimics the activity of the native molecule. IL-3 dependent murine mast cell lines have been established by infecting bone marrow mast cells using AD12-SV40 hybrid virus. Cell lines established are similar to mast cells grown in primary culture from bone marrow. Mast cells synthesize heparan sulfate, collagen IV, and laminin which are the principle components of basement membrane. $FC_{\varepsilon}RI$ crosslinking in primary mast cell cultures results in the induction of multiple cytokine genes; significant levels of RNA for TGF-beta can be found in resting mast cells; cytokine genes can be divided into two groups (pro-inflammatory and growth factors) based on the intensity of the RNA signal and the time course of expression; and the expression of these groups appears to be regulated by distinct mechanisms. Dexamethasone added to cultured mast cells inhibits RNA synthesis.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00514-03 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diagnosis and treatment of Adverse Reactions to Foods and Additives

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Dean D. Metcalfe, M.D. Head, Mast Cell Phsiology Section LCI/NIAID

COOPERATING UNITS *(if any)*

Indian Institute of Science, Bangalore, India (P.V. Subba Rao)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.50	0.25	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have completed data analysis for the clinical study to determine the reproducibility of "allergic" reactions to aspartame, a non-nutritive sweetener. Although efforts to notify potential subjects of this protocol by means of notices in newspapers and information presented to local physicians continued over 2 and 1/2 years, we received only 61 inquiries. Twenty patients were evaluated in clinic. Twelve of these individuals underwent blinded challenge. No subject with a clearly reproducible adverse reaction to aspartame was identified. In summary, we found it difficult to recruit study subjects with a history of allergic reactions to aspartame, and that subjects who believed themselves allergic to aspartame did not have reproducible reactions.

We have completed the initial clinical studies to determine the effect of monosodium glutamate (MSG) on bronchial hyperreactivity. Normal volunteers had no change in pulmonary function upon blind challenge with MSG. Subjects with mild to moderate asthma were next challenged. These individuals also demonstrated stable pulmonary function after MSG.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00521-03 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effect of Cytokines in Host Defense and Inflammation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John I. Gallin, M.D. Director, DIR,	NIAID
Others:	Joan M.G. Sechler	LCI/NIAID
	Harry L. Malech, M.D. Head, Bacterial Dis. Sec.	LCI/NIAID
	Stuart Abramson, M.D, Ph.D. Medical Staff Fellow	LCI/NIAID
	Thomas L. Leto, Ph.D Senior Staff Fellow	LCI/NIAID
	Rachel Levy, Ph.D., Visiting Associate	LCI/NIAID
	Ellen DeCarlo, R.N, Research Nurse	DIR/NIAID

COOPERATING UNITS *(if any)*

T Nutman and C King, LPD/NIAID; K Lomax, J Rex, and J Bennett, LCI/NIAID

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Bacterial Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.1	2.7	0.4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to study effects of cytokines upon host defense and upon phagocytic cell function. Interferon gamma (IFN-g) corrected abnormal oxidative metabolism and bactericidal and fungicidal activity of neutrophils and monocytes from patients with chronic granulomatous diseases of childhood (CGD) whether administered in vivo or in vitro. 127 CGD patients (27 at NIH) were entered into a double blinded study of the efficacy of every other day prophylactic IFN-g (0.05 mg/square-meter) in preventing infection. IFN-g administration decreased the incidence of infection by 70% and decreased hospital days for those infections which did occur. Normal monocytes treated in vitro with IFN-g increased mRNA transcripts and protein levels of membrane and cytoplasmic NADPH oxidase components, while Interleukin 4 (IL-4) markedly decreased these components. The p47-phox oxidase component increased or decreased more than other components, indicating that this component is most critical in cytokine regulation of oxidase activity. IFN-g can induce differentiation of HL-60 myeloid leukemia cells with acquisition of oxidase capacity. During such differentiation the most dramatic increase was seen with p47-phox, while p67-phox appeared most slowly. In other studies B-cells from patients with Hyperimmunoglobulin E and Recurrent Infections (Job's) Syndrome (HIE) were shown to have abnormally high spontaneous in vitro synthesis of IgE which was decreased by administration of IFN-g in vitro or in vivo. Production of IgG1,3, and 4, but not IgG2 or IgM was also decreased by IFN-g. In other studies CGD monocytes in vitro formed granulomas and these granulomas could be dispersed with steroids.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00548-02 LCI

PERIOD COVERED

Octobrt 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Prevention of Genital Herpes Simplex Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S.E. Straus	Senior Investigator	LCI,NIAID
OTHER:	A. Freifeld	Guest Researcher	LCI,NIAID
	J. Dale	Clinical Research Nurse	LCI,NIAID
	B. Savarese	Guest Research Nurse	LCI,NIAID
	D. Paar	Medical Staff Fellow	LCI,NIAID
	D. Margolis	Medical Staff Fellow	LCI,NIAID
	P. Krause	Medical Staff Fellow	LCI,NIAID
	J. Meier	Medical Staff Fellow	LCI,NIAID

COOPERATING UNITS (if any)

R.L.Burke, C.Dekker (Chiron, Inc., Emeryville, CA)
L.Corey (University of Washington, Seattle)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Having completed a decade of work on antiviral treatment of genital herpes, we turned this year to studies of disease prevention. We began with phase 1 assessment of a new recombinant HSV-2 glycoprotein D vaccine in alum. We recruited and vaccinated 24 adults, some with and some without prior HSV-1 and/or 2 infection. ELISA titers to gD, neutralizing antibodies and lymphocyte blastogenesis are being following serially. Vaccinations were well tolerated and induced marked rises or boosts in g-D specific responses. In the coming year we will initiate a placebo controlled trial in subjects with frequent gential recurrences and begin to explore alternative adjuvants in phase 1 studies.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00589-01

PERIOD COVERED
August 1989- July 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Identification of Complement Resistance Determinants in Escherichia coli

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Thomas A. Russo M.D.C.M., Senior Staff Fellow, NIAID, LCI

Michael M. Frank M.D., Senior Investigator, NIAID, LCI
Jane Guenther B.S., Technician, NIAID, LCI

COOPERATING UNITS (if any)

John Foulds Ph.D., Senior Investigator, NIDDK, LSD

LAB/BRANCH Laboratory of Clinical Investigation

SECTION Clinical Immunology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Complement resistance is an important virulence factor in the pathogenesis of bacterial gram negative infections. A more complete understanding of its complement resistance determinants may lead to improved or novel modalities for therapeutic intervention as well as an insight as to the mechanism of complement's action.The experimental approach taken for this project involves transposon mutagenesis of the chromosome and plasmid(s) of a clinical isolate of *Escherichia coli* that is highly complement resistant. We are using a transposon system (TnphoA) designed to mutagenize and identify mutants in outer, periplasmic and cytoplasmic membrane proteins. Mutants are screened for an increased sensitivity to serum and the acquisition of this phenotype will presumably be the result of the disruption of genes that play a role in complement resistance.This method makes no assumptions as to what genes contribute to complement resistance and will hopefully identify previously unrecognized gene products that play a role in this process.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00590-01 LCI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Primary Immunodeficiency Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. Michael C. Sneller, M.D., Senior Staff Fellow, Mucosal Immunity Section, LCI, NIAID

Other Warren Strober, M.D., Head, Mucosal Immunity Section, LCI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this project is to determine the role of abnormal lymphokine/cytokine expression in the immunopathogenesis of common variable immunodeficiency (CVI). During the present period we have identified significant abnormalities in the expression of certain lymphokine genes in a group of patients with CVI. We examined T cell function in CVI by measuring the expression lymphokine genes in mitogen-activated T cells. The results show that T cells from patients with CVI exhibit deficient production of IL-2, IFN-γ, IL-4, and IL-5. These defects are selective since T cells from patients with CVI retain a normal capacity to express IL-2Rα, c-myc and to proliferate. Further studies indicate that the deficient production of IFN-γ by patient T cells is partially due to the abnormality of IL-2 production as the levels of IFN-γ mRNA detected during the initial, IL-2 independent phase of T cell activation were normal and in vitro treatment with recombinant IL-2 was able to normalize IFN-γ production by PHA-stimulated patient cells. Finally, supernatants from PHA-activated cultures of patients PBMCs were deficient in their ability to support Ig secretion by SAC-activated normal B cells suggesting that these T cell abnormalities may contribute to the pathogenesis of this syndrome. These results point to a profound abnormality of T cell function in patients with CVI that may play an important role in the pathogenesis of this syndrome.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00591-01

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of the Murine Homolog for the Human p47-phox Gene

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Karen J. Lomax, M.D. Head, Gene Therapy Unit

Others : Gail Scully, M.D. Medical Staff Fellow
Claudia Lipschultz Technician

COOPERATING UNITS (if any)

none

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Gene Therapy Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this non-clinical, IIDEA project is to identify, clone, and characterize the murine gene corresponding to the human p47-phox gene which codes for the phagocyte protein deficient in autosomal recessive Chronic Granulomatous Disease. No human subjects are involved. An antibody to human recombinant p47-phox detects a protein of nearly identical size in murine bone marrow. DNA analysis of a series of mammalian, avian,and yeast DNA's revealed DNA fragments homologous to the human gene. Primates were most similar and murine DNA less so. Studies of RNA isolated from two murine cell lines IC21 and WEHI-3 detected mRNA transcripts for the murine p47-phox gene of about 2.5-3.0 kilobases in size. To clone this gene, a cDNA library made from mRNA isolated from WEHI-3 cells was screened using a radioactively labelled human p47-phox cDNA probe. Positive clones were purified and characterized. Five clones were identified as being 2.6-2.8 kb in size. These clones were subjected to DNA sequence analysis. Comparison of the murine and human DNA sequence has revealed 80-85% identity between the murine gene and the human gene in the region of the gene coding for protein. In the majority of cases, base differences occur in the third or "wobble" position of the triplet codon and do not result in amino acid changes in the protein. In the coming year, utilizing gene targeting techniques, the murine gene will be mutated <u>in situ</u> by homologous recombination in an embryonal stem (ES) cell line creating a pluripotent cell line deficient in the murine cytosolic protein. These ES cells can be introduced into murine blastocysts to generate mice carrying an abnormal gene. These animals will provide amodel system for studying the inflammatory response and for genetically correcting their defect. This will provide information on potential ways to correct the human genetic defect in the future.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00592-01

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Retroviral Transfer of p47-phox into Murine Cells and Human Myeloid Cell Lines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Karen J. Lomax, M.D.	Head, Gene Therapy Unit/LCI/NIAID
Others:	Charles S. Cobbs	Pre-URTA Fellow/LCI/NIAID
	Scott M. Freeman, M.D.	Fellow, Div. of Cancer Biology and Diag/NCI
	R. Michael Blaese, M.D.	Division of Cancer Biology and Diagnosis/NCI
	Thomas L. Leto, Ph.D.	Senior Staff Fellow/ LCI/NIAID
	Harry L. Malech, M.D.	Head, BDS/LCI/NIAID
	John I. Gallin, M.D.	Director/DIR/NIAID

COOPERATING UNITS (if any)

Cellular Immunology Section/ Division of Cancer Biology and Diagnosis/NCI
Bacterial Diseases Section/LCI/NIAID

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Gene Therapy Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this non-clinical, IIDEA project is to study the feasibility of using retroviral vectors to transfer the gene which is abnormal in autosomal recessive Chronic Granulomatous Disease(p47-phox) into human hematopoietic cells. Development of retrovirus producer cell lines capable of infecting human blood cells will provide the opportunity to study expression of this protein in precursor and differentiated hematopoietic cells. A cDNA for p47-phox containing the protein coding sequence has been cloned into a retroviral vector known as pLXSN. Two murine producer cell lines have been established, one producing an ecotropic retrovirus capable of infecting rodent cells and the other producing amphotropic retrovirus capable of infecting a wide range of mammalian and avian cells. The amphotropic virus containing p47-phox in both normal(sense) and reverse(anti-sense) orientations was used to infect two leukemia cell lines, HL-60 and U937. RNA and protein analysis demonstrated retrovirally transferred p47-phox genes in these cells. In addition, Epstein Barr Virus transformed B lymphocytes from a normal individual and an AR-CGD patient were also infected. These lines also appear to have been transduced by the retroviruses based on protein and RNA analysis.Assays to determine whether the protein made from the transferred gene is functional are still in progress. EBV transformed B lymphocyte cell lines derived from AR-CGD patient cells may be helpful in studies of correction of the defect since these cells in normals produce a small amount of superoxide and can be studied at the functional as well as molecular level. Expression of transferred p47-phox in murine bone marrow and hematopoietic reconstitution of an irradiated mouse will be necessary before primate and human studies can proceed. Preliminary experiments indicate that it is possible to transfer this gene into murine bone marrow.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00593-01

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mediator Release Resulting from Induced Hereditary Angioedema Swelling

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Arthur Thomas Waytes, M.D., Medical Staff Fellow, LCI, NIAID
Other: Michael M. Frank, M.D., Chief, LCI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1	1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hereditary Angioedema (HAE) is characterized by recurrent episodes of edema, believed to result from a marked decrease in functional C1 inhibitor (C1 INH). The pathologic changes are primarily due to vascular leakage into the subepithelium. Identification of the actual mediator(s) responsible, however, has thus far eluted investigators. Presently, bradykinin and a cleavage fragment of C2, "C2-kinin", are believed to be the most likely candidates. Recent work in this laboratory has demonstrated that cleavage of a sialoglycoprotein, sgp 120, by kallikrein results in the release of a small molecular weight fragment which is also a potent mediator of vascular permeability. The objective of this project is to identify mediator(s) involved in HAE swelling.

Much of our initial work was performed developing a sensitive ELISA that enables us to directly measure trace quantities of small molecular weight fragments released from sgp 120, high molecular weight kininogen (including bradykinin), and C2 from human plasma. Blood is obtained with or without plasma protein inhibitors and the plasma or serum is spun through filters which separate released fragments with a molecular weight of less than 10,000, from larger plasma proteins. These fragments are detected with specific polyclonal or monoclonal antisera. We have observed that sera and plasma obtained from HAE patients are more likely to generate small molecular weight fragments of sgp 120 than those obtained from normal individuals. Furthermore; preliminary studies suggest that plasma obtained from HAE patients during an attack may contain increased levels of the sgp 120 fragments than before or after the attack.

To systematically study the release of mediators during an HAE attack, we have designed a protocol, which was approved by the NIAID-CRS, in which HAE patients will be admitted to the Clinical Center for controlled induction of extremity swelling. Blood obtained at established time intervals will be evaluated, using the assay described above, for the presence of small molecular weight fragments released from sgp 120, high molecular weight kininogen, and C2.

LABORATORY OF IMMUNOGENETICS
1990 Annual Report
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LABORATORY OF IMMUNOGENETICS

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

October 1, 1989 to September 30, 1990

RESEARCH PROGRESS

Programs in the Laboratory of Immunogenetics continue to emphasize the gene families that are important to control of immune function. In addition, studies concerning the role of retroviruses that exercise deleterious effects on the immune system are in progress. These studies utilize basic methodology in molecular biology, genetics and immunology. In the past year the laboratory of Dr. P. Baker has joined LIG adding studies on cellular control of immune responses.

Studies on the Human T-cell Receptor Genes

Inheritance patterns of HLA and T cell receptor (TCRα and TCRβ) genes which are known to play important roles in a variety of immune processes have been analyzed in human families. The extent of the TCR repertoire was investigated by analysis of TCR α and β specific cDNA libraries prepared from RNA samples derived from PHA-stimulated peripheral T lymphocytes using a technique involving the Polymerase Chain Reaction (PCR). Clones were obtained that correspond to 21 different TCRβ and 25 different TCRα variable (V) gene families: these include 4 Vβ and 6 Vα families that have not been previously described.

Genetic variation in TCR genes was examined by (i) Southern blot analysis of fragments separated by both conventional and pulsed field gel electrophoresis (PFG) using specific DNA probes to identify restriction fragment length polymorphisms (RFLP); (ii) non-denaturing acrylamide gel electrophoresis to identify single stranded conformational polymorphisms (SSCP) and, (iii) direct sequence comparisons of TCR V region genes. All techniques reveal limited polymorphism in TCR gene segments (**Robinson**). A single nucleotide substitution identified in the Vβ1 gene resulted in an amino acid substitution located within a hypervariable region, whereas single nucleotide substitutions in both Vβ12 and Vα22 genes were found to be conservative. In contrast to the limited polymorphism observed with individual gene segments. There is considerable polymorphism in the combination of markers inherited together in both TCRα and TCRβ haplotypes. In addition, variability in TCR gene complexes derives from the insertion or deletion of segments of DNA; two frequently occurring insertion/deletion related polymorphisms IDRP) were found in the TCRβ complex. The locations of the IDRP were determined by the development of an extended map of the TCRβ complex showing that one involved a stretch of ~30 kb among the V region genes and another spans ~15kb near the C region. Analysis of genes within the TCRα complex begin to give an appreciation of the complexity of this locus (**Grier, Mitchell**).

Analysis of 40 sibling pairs concordant for the relapsing-remitting form of Multiple Sclerosis (MS) revealed that a gene within the TCRβ complex or a closely linked locus influences susceptibility to MS (**Hauser, Robinson**). Studies are in progress to determine whether genes encoding the α chain of the TCR have a similar influence on susceptibility and to determine if families with the implicated TCR genes are susceptible to other autoimmune diseases.

Studies of Human Class II MHC Genes and Products

An immune response occurs only if a T lymphocyte has been able to recognize the foreign antigen in association with a self molecule encoded by the major histocompatibility complex (MHC). These molecules expressed on antigen-presenting cells are the polymorphic class II MHC antigens. Most foreign antigens must be processed in order to bind to MHC molecules. Antigen processing for presentation by class II molecules generally involves endocytosis of antigen into an acidic compartment through which newly synthesized class II molecules are passing on their way to the cell surface. Once at the cell surface, the foreign peptide/MHC class II complex interacts with the T cell receptor and the CD4 molecule expressed on the antigen-specific T cell. The aim of these investigations is to define the function of human class II molecules in their interaction with T cells and the requirements for class II-restricted processing and presentation of viral antigens to CD4-positive T cells.

It has been demonstrated that a cytosolic antigen was endogenously processed in infected cells for class II-mediated presentation. Most interestingly, the processing pathway involved was different from the one utilized for presentation by class I molecules. This unsuspected presentation of endogenous proteins by class II molecules has important implications on the T cell repertoire selection, on T cell tolerance, and on autoimmunity (**Jaraquemada, Malnati, Long, Rivero**).

HLA-DP and HLA-DR are two isotypes of human class II molecules with distinct α and β chains. To define what determines α/β chain assembly, chimeric α and β chains were expressed in a transient expression system. The data showed that the lack of mixed isotype expression was due to a lack of intracellular pairing, and that DP and DR molecules had distinct structural requirements for pairing. Several staphylococcal toxins bind to class II molecules and have a strong mitogenic effect on T cells stimulating families of T cells with particular Vβ chains of the TCR. Using a direct binding assay and chimeric DP/DR molecules expressed by transient tranfection, it was shown that the toxic shock syndrome toxin does not bind detectably to the DP molecule and that the α1 domain of DR is essential for high affinity binding (**Karp, Teletski**).

Use of nontoxic bacterial lipopolysaccharide as an immunomodulator and antagonist

Treatment with the non toxic lipopolysaccharide of *Rhodopseudomonas sphaeroides* (Rs-LPS) or with monophosphoryl lipid A (MPL) have similar effects on the magnitude of the antibody response to Type III pneumococcal polysaccharide (SSS-III). Both appear to augment the magnitude of the antibody response by abrogating the inhibitory effects produced by suppressor

T cells (Ts); other T cell functions are not effected by these immunomodulators. The capacity of Rs-LPS to block the induction of tumor necrosis factor (TNF) by toxic preparations of LPS suggests that nontoxic Rs-LPS might be used prophylactically to prevent the development of endotoxin shock during the early stages of Gram-negative sepsis, as well as to augment the development of increased host immunity, thereby decreasing the total amount of endotoxic likely to be produced during sepsis (**Baker, Haslov and Taylor**).

Enrichment of regulatory T cell activity

Direct evidence was obtained to indicate that the magnitude of the antibody response of mice to bacterial (Leuconostoc) dextran B1355 is influenced in a negative and positive manner by the action of suppressor T cells (Ts) and amplifier T cells (T_A), respectively. Thus, the antibody response to this well-defined antigen can be added to the growing list of polysaccharide antigens, e.g., Type III pneumococcal polysaccharide (SSS-III), *Neisseria meningitidis* group A and C capsular polysaccharides, *Pseudomonas aeruginosa* lipopolysaccharide, *Streptococcus mutans* polysaccharide, and *Serratia marcesans* lipopolysaccharide, whose antibody responses are known to be influenced by regulatory T cells (Ts and T_A). Evidence was obtained to indicate that populations of these regulatory T cells expand in a clonal manner in response to immune B cells and that such expansion is facilitated by IL-2.

Suppressor and amplifier T cells (Ts and T_A), once activated, appear to acquire a cell surface receptor, capable of binding to monophosphoryl lipid A (MPL). Because of differences in the time after immunization when these regulatory T cells first become activated and reach maximal activity during the antibody response to Type III pneumococcal polysaccharide (SSS-III), it is possible to utilize the binding and subsequent elution of such cells from plastic dishes coated with MPL to obtain cell suspensions, greatly enriched (>1,000-fold) in antigen-specific Ts activity. Similar results were obtained for Ts involved in the antibody response to the a1,3 determinant of bacterial (Leuconostoc) dextran. The ability to obtain cell suspensions greatly enriched in Ts activity should enable one to obtain more precise information on the mechanisms by which regulatory T cells produce their effects. (**Baker, Taylor and Haslov**).

Infection of Rabbits with HIV-1

Studies are in progress to develop the laboratory rabbit as a model system for infection with the human retrovirus HIV-1 and to further study the effects of HTLV-I on this animal.

It was previously observed that injection of rabbits a the human T cell line infected with HIV-1 causes seroconversion within four weeks of injection. In the present studies, we have used the polymerase chain reaction in order to detect viral sequences in cells and organs from infected rabbits at various times following the single injection that causes HIV-1 infection (**Truckenmiller**). In one experiment rabbits were sacrificed at two week intervals following infection and various organs were taken, DNA was isolated and monitored for the presence of sequences from the gag and env regions of HIV-1. Results thus far show that animals sacrificed two weeks after injection are negative in all organs for HIV-1 sequences. After four weeks, the spleen is the most likely organ to be positive for HIV-1 followed by lung

and appendix. The liver was least likely to be positive. DNA isolated from peripheral blood lymphocytes taken from animals prior to 10 weeks post-infection was almost always negative for viral sequences. In other studies, brains were taken from animals at 3.5 and 6 months following infection and dissected into various regions. RNA isolated from these regions was then tested by RNA PCR. It was found that the thalamus was the most common site of viral transcript (**Kulaga, Recker**). All animals, however, were positive in at least two of the regions tested for both gag and env sequences of HIV-1.

In order to determine the effect of HIV infection on immune function in the rabbit, the immune responses to tetanus toxoid and BCG were tested in rabbits that had been infected with HIV-1 (**Gordon**). Various protocols were tried and only one produced significant evidence for immune suppression. When animals were immunized and one week later were infected with HIV-1, these animals seemed to have significantly diminished humoral and cellular responses. The cellular responses were measured by skin tests and by lung granulomatosis index following sacrifice 6 months after immunization.

It was earlier shown that supernatants taken from rabbit macrophages infected with HIV-1 were negative for reverse transcriptase (RT) activity. In these cells other parameters for infection were positive. The activity giving rise to the RT negativity was shown to be a nuclease that can digest either substrates or products of the assay system (**Recker**). Attempts are being made to circumvent this activity in order to be able to use RT as a measure of cell infection. In addition to finding this activity in the rabbit macrophage line, it has also been found in the human cell line U937 and in adherent macrophages from both rabbit and human peripheral blood.

Rabbit MHC Genes

Recent studies on the major histocompatibility complex (RLA) of the rabbit have developed reproducible methods to type rabbits for MHC genes. Current studies are using animals with known MHC types in order to determine the effect of the RLA antigens on immune responsiveness and basic studies of MHC gene structure have continued emphasizing sequence analysis of DQα class II genes from the rabbit.

Investigations are underway in which cellular responses to certain proteins are being tested using cells from MHC typed rabbits. These studies center about development of immunity to structural and regulatory proteins of the human retrovirus HIV-1. Antibody and cellular immune responses to the envelope protein gp160 and to the regulatory protein nef are being studied (**LeGuern**). Immunity to these proteins have been elicited by injection with either protein or derived peptides and responses to both protein and peptide are being tested. Rabbits having cellular and humoral responses that neutralize virus will be used for challenge with live HIV-1 in an attempt to determine which of these immunization procedures provide protection against virus infection.

A number of antibodies have been used in an attempt to characterize functionally distinct rabbit lymphoid cell populations, to identify cell surface markers for them and to relate these populations to those that are found in humans. Various techniques for peripheral blood cell

isolation have been tried in order to obtain preparations of peripheral blood cells that are uniform with respect to distribution of cell type and also with respect to their response to stimulation with various T and B cell mitogens.

A monoclonal antibody, 93C6, that reacts with activated T cells has been identified and its antigen has been found on spleen and appendix cells but not on thymus. Thymus cells become positive for 93C6 when stimulated with T-cell mitogens. Preliminary results suggest that the antigen of 93C6 may be a component chain of the interleukin-2 receptor. A second monoclonal antibody, M110, was found to react with a small subset of cells from peripheral blood in the rabbit and with certain rabbit T cell lines prepared by transformation with HTLV-1. The antigen reacting with M110, is currently being sought and its cell distribution is being examined in various lymphoid organs of the rabbit (**Gordon**). Attempts to characterize a CD4 homolog to serve as a marker for the T helper subset of rabbit lymphocytes have not thus far been successful. Current attempts include amplification of both DNA and RNA samples using PCR primers with sequence based on regions of CD4 conserved among the human, rat and mouse molecules (**Lee**).

In vitro and *in vivo* effects of HTLV-I on rabbits

Studies were carried out using HTLV-1 as a transforming agent for rabbit cells. Several methods have been successfully used to derive cell lines that have markers characteristics of T cells. These lines are currently being used to characterize and isolate the interleukin-2 receptor of the rabbit and are being used as targets to study HIV-1 infection in the rabbit. In addition to studies of the HTLV-1 cell line in vitro, rabbits infected with the HTLV-1 infected human cell line, MT2, are being studied to determine whether the infection has any effect on circulating lymphoid cell populations or on immune function (**Sawasdikosol**).

It is not known what receptor(s) on rabbit cells bind to HIV-1. Studies to determine the effect of CD4 on infection have shown that soluble human CD4 will block infection of certain cells. Recently a line has been derived by transfection of the cell line RL-5 with a gene encoding human CD4 (**Hague**). The transfectants were infected with HIV-1 but showed no substantial increase in any parameter associated with HIV-1 infection. However, when supernatants from the MT2 cell line (a human cell line infected with HTLV-I) were placed on the transfected RL-5, there was evidence for extensive infection of the transfected cell line with HTLV-1; when the transfectant cells were examined by electromicroscopy it was shown that a large percentage of the transfected cells produced virus like particles and, in addition, the virus appears to be cytopathic which is not normal for HTLV-1 on rabbit cells.

LABORATORY OF IMMUNOGENETICS

ANNUAL REPORT

October 1, 1989 to September 30, 1990

HONORS AND AWARDS

During the past year, Dr. Thomas Kindt was an invited speaker at the 37th National Convention of Child Care Executives in Laramie, Wyoming; at the Second International Conference on Drug Research in Immunologic and Infectious Diseases, in Arlington, VA, the UCLA Colloquium on Animal Models for Viral Disease, Keystone, CO, and at the conference on Human Antibodies: Genes and Expression in Autoimmune Disease meeting in Annecy, France. Laboratory data were presented at the annual meeting of the Laboratory of Tumor Cell Biology; at Ciba-GEIGY Limited and at the Basel Institute for Immunology, Basel, Switzerland, at the Pasteur Institut, Paris; at the Institute for Microbiology in Genoa, Italy, at ImmuLogic Pharmaceutical Corp and Biogen Inc, Cambridge, MA and at Monsanto Corporation in St. Louis. Dr. Kindt also presented data at the conference on AIDS in Mothers and Children in Paris, France. Dr. Kindt continues to serve on the review board for the Multiple Sclerosis Society, Israel Cancer Research Fund, on the Board of Scientific Visitors for the Oklahoma Medical Reserach Foundation and as an advisor to students for the Howard Hughes Medical Institute. He serves as a scientific advisor to Oncor Inc and to Southern Biotechnology, Inc and was appointed to the scientific board of Innovir Inc. Dr. Kindt also serves as a deputy editor of the Journal of Immunology and is on the board of the Journal of Experimental Medicine, and is a regional editor for Research in Immunology. Dr. Kindt was elected to the nominations committee of the American Association of Immunologists and was awarded the Superior Service Award from the Assistant Secretary DHHS.

Dr. Eric Long presented lectures at McGill University, Montreal, at the University of Massachusetts Medical School, at the Ontario Cancer Institute and Department of Immunology, University of Toronto, the Royal Postgraduate Medical School, Hammersmith Hospital, London, at the Cancer Research Institute's workshop on "Cellular routes of antigen processing and presentation", at the EMBL, Heidelberg, the University of Geneva Medical School, Geneva and at the Ludwig Institute for Cancer Research, Lausanne. Dr. Long was also an invited speaker at the 7th International HLA/H-2 workshop in Munich, at the annual meeting of the South-Eastern Organ Procurement Foundation in Toledo, Ohio, at the Dageraad Symposium in Enkhuizen, The Netherlands and at NIH Research Day. He serves as an Associate Editor for the Journal of Immunology and served as a guest editor for Immunologic Research for a symposium on MHC class II genes and molecules. Assumed chair of the NIAID Animal Care Users Committee.

Dr. Phillip Baker presented lectures at the University of Maryland and at George Washington University School of Medicine. He is an Editor for Infection and Immunity and a member of the Editorial Board for the ASM News. He is Councilor of the Immunology Division of the American Society for Microbiology (ASM) and also serves on the Public Relations Committee of the ASM. He was invited to present a paper, as well as to chair a session, at the Congress of the International Endotoxin Society (IES). He is on the Publications Committee of the IES and was nominated for the position of Councilor of the IES. He presented a paper and convened a

symposium at the Annual Meeting of the ASM. He was invited to be the keynote speaker, as well as to convene a session, at the International Symposium on the "Epidemiology, Pathogenesis and Prevention of *Haemophilus influenzae* Disease" in Veldhoven, The Netherlands.

Dr. Christopher Taylor gave lectures at Howard University School of Medicine and at the George Washington University School of Medicine; he presented two papers at the FASEB meeting and was invited to present a paper at the International Symposium on the "Epidemiology, Pathogenesis and Prevention of *Haemophilus influenzae* Disease" in Veldhoven, The Netherlands.

Dr. Mary Ann Robinson presented invited lectures at Emory University, North Shore University Hospital, Cornell University Medical College and at the Walter Reed Medical Center and presented current laboratory data at the ASHI meeting in October. Dr. Robinson served on the Abstract Awards and Selection Committee for the ASHI meeting. She also served as an Adhoc Reviewer for the United States-Israel Binational Science Foundation. Dr. Robinson serves on the NIAID Safety Committee, Introduction to Biomedical Research Selection committee and is a consultant for the Howard University College of Medicine Research Center on Minority Institutions Program.

LABORATORY OF IMMUNOGENETICS
Annual Report
October 1, 1989 to September 30, 1990

ADMINISTRATIVE REPORT

Dr. David Recker a Medical Staff Fellow in the Immunogenetics Research Section, will depart to accept a position at the Office of Technology Assessment in Washington, D.C. Dr. Kevin Lee, a NRC fellow, will take a research position at the University of Pittsburgh. Dr. Alice Grier,an IRTA fellow, is departing to assume a faculty position at Washington and Jefferson College in Pennsylvania. Dr. Camilla Day from Rutgers University will join LIG in mid August as will Dr. Lotfi Chouchane from the Hopital Cochin in Paris; both will have positions in the immunogenetics research section. In the Molecular Immunology Section, Dr. Dolores Jaraquemada, who has served as a Visiting Fellow, is leaving to take a position at the Unitat D'Immunologia in Barcelona, Spain. Ms. Merci Marti, a Guest Researcher, will depart to become a student in Dr. Jaraquemada's lab in Spain. Dr. Jose Rivera from Argentina will join the Molecular Immunology Section of LIG. In the Microbial and Immunology Section, Dr. Kaare Haslov, a Visiting Associate, will return to Denmark to assume a faculty position. Dr. Thomas Hraba, a distinguished scientist from Prague, Czechoslovakia, will join that section as a Visiting Scientist early in the next year.

It is planned that the Laboratory of Immunogenetics will abandon its space in Building 4 on the NIH campus and move in its entirety to the Twinbrook II facility in Rockville, Maryland. Dr. Baker's section is currently at the Twinbrook II facility.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00143-21 LIG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Control of the Antibody Response to Microbial Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.J. Baker, Head	LIG, NIAID	Microbiology & Immunology Section
OTHERS:	P.W. Stashak	LIG, NIAID	Microbiologist
	M.B. Fauntleroy	LIG, NIAID	Biologist
	K.L. Holmes	BRB, NIAID	Senior Investigator

COOPERATING UNITS *(if any)*

J. Hiernaux, GLAXO Ltd, Paris; EA Goidl and SJM McEvoy, Dept. of Microbiology and Immunology, University of Maryland, School of Medicine, Baltimore, MD 21201

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Microbiology and Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Twinbrook-II Res. Facility, 12441 Parklawn Dr, Rockville, MD 20852

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

RIIIS/J mice lack an autosomal dominant gene(s) that influences the magnitude of the antibody response to several bacterial polysaccharide antigens. Low responsiveness is demonstrable, whether or not polysaccharide is administered as a helper T cell-independent antigen or as a helper T cell-dependent antigen conjugated to an immunogenic carrier; however, RIIIS/J mice make good anti-hapten antibody responses to haptenated polysaccharides. The low antibody response of RIIIS/J mice to Type III pneumococcal polysaccharide (SSS-III) does not appear to be due to an imbalance in the activity of regulatory T lymphocytes. In comparison to other strains of mice, RIIIS/J mice elicit low antibody responses to bacterial lipopolysaccharide (LPS). They do not develop a cyclic primary antibody response or a secondary antibody response to LPS; the latter is not due to the lack of a mitogenic response to LPS. They also produce auto-anti-idiotypic antibody after immunization with TNP-Ficoll.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00144-26 LIG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of the Antibody Response to Microbial Polysaccharide Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P.J. Baker, Head	LIG, NIAID	Microbiology & Immunology Section
OTHERS:	C.A. Taylor	LIG, NIAID	Senior Staff Fellow
	K.R. Haslov	LIG, NIAID	Visiting Associate
	P.W. Stashak	LIG, NIAID	Microbiologist
	M.B. Fauntleroy	LIG, NIAID	Biologist

COOPERATING UNITS (if any)

JA Rudbach and JT Ulrich, Ribi ImmunoChem Res. Inc., Hamilton, MT; KT Takayama, Mycobacteriology Lab. Middleton Mem. VA Hospital, Madison, WI; KL Elkins, Dept. Cellular Immunology, Walter Reed Army Institute of Research, Washington, DC 20307

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Microbiology and Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Twinbrook-II Res. Facility, 12441 Parklawn Dr, Rockville, MD 20852

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.4	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The binding and elution of spleen cells from plastic dishes coated with monophosphoryl lipid A (MPL) resulted in a >1,000-fold enrichment of antigen-specific suppressor T cell (Ts) activity when spleen cells taken from mice 18-24 hr after exposure to a low dose of Type III pneumococcal polysaccharide (SSS-III) were used. The removal of MPL-adherent Ts cells resulted in an increase in the degree of amplifier T cell (T_A) activity present in the remaining MPL-nonadherent cell fraction; however, both Ts and T_A activities were found in the MPL-adherent cell fraction when spleen cells from mice 4 days after immunization with an optimal dose of SSS-III were examined. These findings, as well as others, suggest that both Ts and T_A - once activated - acquire a cell surface receptor that enables them to bind to MPL. Because of differences in the kinetics for the activation of Ts and T_A during the course of the antibody response and the fact that Ts - but not T_A - activity appears as early as 18-24 hrs after exposure to SSS-III, it is possible to use this experimental approach to obtain cell suspensions greatly enriched in Ts activity.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00145-23 LIG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mode of Action of Thymus-derived (T) Suppressor and Amplifier Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.J. Baker, Head	LIG, NIAID	Microbiology & Immunology Section
OTHERS:	C.A. Taylor	LIG, NIAID	Senior Staff Fellow
	K.R. Haslov	LIG, NIAID	Visiting Associate
	P.W. Stashak	LIG, NIAID	Microbiologist
	M.B. Fauntleroy	LIG, NIAID	Biologist

COOPERATING UNITS *(if any)*

JA Rudbach, Ribi ImmunoChem Research, Inc., Hamilton, MT; KT Takayama, Mycobacteriology Lab, William S. Middleton Memorial VA Hospital, Madison, WI; KL Elkins, Depart. Cellular Immunology, Walter Reed Army Institute of Research, Washington D.C. 20307

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Microbiology and Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Twinbrook-II Res. Facility, 12441 Parklawn Dr, Rockville, MD 20852

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	1.5	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Direct evidence was obtained to indicate that the magnitude of the antibody response of mice to bacterial (Leuconostoc) dextran B1355 is influenced in a negative and positive manner by the action of suppressor T cells (Ts) and amplifier T cells (T_A), respectively. Thus, the antibody response to this well-defined antigen can be added to the growing list of other microbial polysaccharide antigens, e.g., Type III pneumococcal polysaccharide (SSS-III), *Neisseria meningitidis* group A and C capsular polysaccharides, *Pseudomonas aeruginosa* lipopolysaccharide, *Streptococcus mutans* polysaccharide, and *Serratia marcesans* lipopolysaccharide, whose antibody responses are known to be influenced by regulatory T cells (Ts and T_A). Evidence was obtained to indicate that populations of these regulatory T cells expand in a clonal manner in response to immune B cells and that such expansion is facilitated by IL-2.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00166-13 LIG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Characterization of Rabbit MHC Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas J. Kindt	Chief	LIG, NIAID
OTHER:	A. LeGuern	Visiting Associate	LIG, NIAID

COOPERATING UNITS *(if any)*

Patrice Marche, Institut Pasteur, Paris; Christian LeGuern, Immunol Br., NCI; Marc Girard, Pasteur Vaccins, Paris

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Earlier studies have provided molecular methods by which rabbit MHC antigens can be conveniently typed. The goal of the present study is to use animals with known MHC types in order to determine the effect of these antigens on immune responsiveness. In addition, basic studies of MHC gene structure have continued emphasizing sequence analysis of DQα class II genes from the rabbit. Several studies are underway in which cellular responses to certain proteins are being tested using MHC typed rabbit cells. These studies center about development of immunity to structural and regulatory proteins of the human retrovirus HIV-1. Antibody and cellular immune responses to the envelope protein gp160 and to the regulatory protein nef are being studied. Immunity to these proteins has been elicited by injection of either protein or peptide and responses to the protein and peptide are being tested. Rabbits having cellular and humoral responses that neutralize virus will be used for challenge with live HIV-1 in an attempt to determine whether these immunization procedures provide protection against virus infection.

PHS 6040 (Rev. 1/84) GPO 814-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00168-13 LIG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cell Surface Markers of Rabbit Lymphocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Thomas J. Kindt	Chief	LIG, NIAID
OTHERS:	K. Lee	NRC Fellow	LIG, NIAID
	Mark Gordon	NRC Fellow	LIG, NIAID

COOPERATING UNITS (if any)

Mike Roy, Walter Reed Research Institute.

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A number of antibodies have been used in an attempt to functionally characterize distinct rabbit lymphoid cell populations, to identify cell surface markers for them and to relate these populations to those that are found in humans. These antibodies have come from a variety of sources including immunization with rabbit proteins and lymphoid cell lines, from commercial sources and from other investigators in this field. Various techniques for peripheral blood cell isolation have been tried in order to obtain preparations of peripheral blood cells that are uniform with respect to distribution of cell type and also with respect to their response to stimulation with various T- and B-cell mitogens. An antibody, 93C6, that reacts with activated T cells has been identified and its antigen has been found on spleen and appendix cells but not on thymus. Thymus cells become positive when stimulated with a T cell mitogen. Preliminary results suggest that the antigen of 93C6 may be a component chain of the interleukin-2 receptor. A second monoclonal antibody, M110, was found to react with a small subset of cells from peripheral blood in the rabbit and with certain rabbit T cell lines prepared by transformation with HTLV-1. The antigen recognized by M110, is currently being sought and its cell distribution is being examined in various lymphoid organs of the rabbit. Attempts to characterize a CD4 homolog to serve as a marker for the T helper subset of rabbit lymphocytes has not thus far been successful. A number of attempts at both the protein and nucleic acid level have not yielded a likely candidate. Current attempts include the use of various PCR primers based on regions of the CD4 sequences conserved among the human, rat and mouse molecules.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00170-13 LIG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular and Functional Analysis of Human Class II Histocompatibility Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. O. Long	Visiting Scientist	LIG, NIAID
OTHERS:	D. R. Karp	Senior Staff Fellow	LIG, NIAID
	D. Jaraquemada	Visiting Fellow	LIG, NIAID
	M. Jendoubi	Visiting Associate	LIG, NIAID
	M. Malnati	Visiting Fellow	LIG, NIAID
	J. L. Rivero	Visiting Fellow	LIG, NIAID
	C. Teletski	Microbiologist	LIG, NIAID

COOPERATING UNITS (if any)

H. McFarland,Neuroimmunology Branch, NINCD, NIH; R. Sekaly, Clinical Research Institute of Montreal, Canada; R. Geha, Children's Hospital, Boston, MA; S. Rosen-Bronson, Dept. of Pediatrics, Georgetown University.

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Molecular Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.5	5	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An immune response occurs only if a T lymphocyte has been able to recognize the foreign antigen in association with a self molecule encoded by the major histocompatibility complex (MHC). These molecules expressed on antigen-presenting cells are the polymorphic class II MHC antigens. Most foreign antigens must be processed in order to bind to MHC molecules. Antigen processing for presentation by class II molecules generally involves endocytosis of antigen into an acidic compartment through which newly synthesized class II molecules are passing on their way to the cell surface. Once at the cell surface, the foreign peptide/MHC class II complex interacts with the T cell receptor and the CD4 molecule expressed on the antigen-specific T cell. The aim of this project is to define the function of human class II molecules in their interaction with T cells and the requirements for class II-restricted processing and presentation of viral antigens to CD4-positive T cells. It has been demonstrated that a cytosolic antigen was endogenously processed in infected cells for class II-mediated presentation. Most interestingly, the processing pathway involved was different from the one utilized for presentation by class I molecules. This unsuspected presentation of endogenous proteins by class II molecules has important implications on the T cell repertoire selection, on T cell tolerance, and on autoimmunity. HLA-DP and HLA-DR are two isotypes of human class II molecules with distinct α and β chains. To define what determines α/β chain assembly, chimeric α and β chains were expressed in a transient expression system. The data showed that the lack of mixed isotype expression was due to a lack of intracellular pairing, and that DP and DR molecules had distinct structural requirements for pairing. Several staphylococcal toxins bind to class II molecules and have a strong mitogenic effect on T cells stimulating families of T cells with particular Vβ chains of the TCR. Using a direct binding assay and chimeric DP/DR molecules expressed by transient tranfection, it was shown that the toxic shock syndrome toxin does not bind detectably to the DP molecule and that the alpha1 domain of DR is essential for high affinity binding.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00171-13 LIG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Infection of Rabbits with Human Immunodeficiency Virus 1

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Thomas J. Kindt	Chief	LIG, NIAID
OTHERS:	M Gordon	Staff Fellow	LIG, NIAID
	D R Recker	Medical Staff Fellow	LIG, NIAID
	M E Truckenmiller	IRTA	LIG, NIAID

COOPERATING UNITS (if any)

Thomas Folks, CDC, Atlanta; Alan Lock, Div. Vet Path., NIH; Henrietta Kulaga, Neuropsychiatry Br., NIMH

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	2.5	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It was previously observed that injection of rabbits with a human T cell line infected with HIV-1 causes seroconversion within four weeks of injection. In the present studies, we have used the polymerse chain reaction in order to detect viral sequences in cells and organs from infected rabbits at various time points following the single injection that causes HIV-1 infection. In one group of rabbits, animals were sacrificed at two week intervals following infection and various lymphoid and other organs were taken, DNA was isolated and monitored for the presence of sequences from the gag and env regions of HIV-1. In other experiments, animals were super-infected with HTLV-1 approximately one year after HIV-1 infection and peripheral blood and lymphoid organs were monitored at various time intervals following the super-infection event. Results thus far show that animals sacrificed two weeks after injection are negative in all organs for HIV-1 sequences. After four weeks, the spleen is the most likely organ to be positive for HIV-1 followed by lung and appendix. The liver is least likely to be positive. DNA isolated from peripheral blood lymphocytes taken from animals prior to 10 weeks post-infection was almost always negative for viral sequences. After this time, there was the higher likelihood to detect virus. In other studies, brains were taken from animals at 3.5 and 6 months following infection and dissected into various regions. RNA isolated from these regions was then tested by RNA PCR. It was found that the thalamus was the most common site of viral transcript. All animals, however, were positive in at least two of the regions tested for both gag and env sequences of HIV-1. In other experiments, the immune responses to tetanus toxoid and BCG were tested in rabbits that had been infected with HIV-1. Various protocols were tried and only one produced significant evidence for immune suppression. Animals that were immunized and one week later infected with HIV-1 had significantly diminished humoral and cellular responses. The cellular responses were measured by skin tests and by lung granulomatosis index following sacrifice 6 months after immunization.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00173-12 LIG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Control of Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: E. E. Max

COOPERATING UNITS (if any)

Laboratory of Immunogenetics

LAB/BRANCH

Immunogenetics Research Section

SECTION

NIAID, NIH, Bethesda, Maryland

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00180-12 LIG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Properties of Transformed Rabbit Cell Lines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Thomas J. Kindt	Chief	LIG, NIAID
OTHER:	B Hague	IRTA	LIG, NIAID
	D R Recker	Medical Staff Fellow	LIG, NIAID
	S Sawsodikosol	Biologist	LIG, NIAID

COOPERATING UNITS (if any)

Chamer Wei and Frances Gillespie, Transgenic Sciences, Inc., MA

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies were carried out using HTLV-1 as a transforming agent for rabbit cells. Several methods have been successfully used to derive cell lines that have markers characteristics of T cells. These lines are currently being used to characterize and isolate the interleukin-2 receptor of the rabbit and are being used as targets to study HIV-1 infection in the rabbit. In addition to studies of the HTLV-1 cell line <u>in vitro</u>, rabbits infected with the HTLV-1 infected human cell line, MT2, are being studied to determine whether the infection has any effect on circulating lymphoid cell populations or on immune function. Certain rabbit cell lines serve as targets for HIV-1 infection, a new line has been derived by transfection of the cell line RL-5 with a gene encoding human CD4. The transfectants were infected with HIV-1 but showed no substantial increase in any parameter related to HIV-1 infection. However, when supernatants from the MT2 cell line were placed on the transfected RL-5, there was evidence for extensive infection of the transfected cell line with HTLV-1; the parent line was examined by electromicroscopy and it was shown that a large percentage of the transfected cells produced virus like particles and, in addition, the virus appeared to be cytopathic which is not normal for HTLV-1 on rabbit cells. The protein produced by the HTLV-1 infected, transfected cells, as well as cells doubly infected with both HTLV-1 and HIV-1, are now being examined to determine whether there are pseudotype viral particles being produced by the transfected line. In other experiments, it had been shown that supernatants taken by rabbit macrophages infected with HIV-1 were negative for reverse transcriptase (RT) activity. In these cells other parameters for infection were positive. The ;activity giving rise to the RT negativity was shown to be a nuclease that can digest either substrates or products of the assay system. Attempts are being made to circumvent this activity in order to be able to use RT as a measure of cell infection. In addition to finding this activity in the rabbit macrophage line, it has also been found in the human cell line U937 and in adherent macrophages from both rabbit and human peripheral blood.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00389-07 LIG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genotype Analyses of HLA and TCR Genes in Human Families

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M.A. Robinson	Expert	LIG, NIAID
OTHERS:	A. Grier	IRTA	LIG, NIAID

COOPERATING UNITS (if any)

D. Bernard Amos, Duke University, S. Hauser, Massachusetts General Hospital; D. Posnett, Cornell University Medical Center; A. A. Ansari and G. Rodey, Emory University.

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Inheritance patterns of HLA and T cell receptor (TCRα and TCRβ) genes which are known to play important roles in a variety of immune processes have been analyzed in human families. The extent of the TCR repertoire was investigated by analysis of TCR α and β specific cDNA libraries prepared from RNA samples derived from PHA-stimulated peripheral T lymphocytes using a technique involving the Polymerase Chain Reaction (PCR). Clones were obtained that correspond to 21 different TCRβ and 25 different TCRα variable (V) gene families including 4 Vβ and 6 Vα families that have not been described previously. Genetic variation in TCR genes was examined by (i) Southern blot analysis of both conventional and pulsed field gels (PFG) using specific DNA probes to identify restriction fragment length polymorphisms (RFLP); (ii) non-denaturing acrylamide gel electrophoresis to identify single stranded conformational polymorphisms (SSCP) and, (iii) direct sequence comparisons of TCR V region genes. All techniques reveal limited polymorphism in TCR gene segments. A single nucleotide substitution identified in the Vβ1 gene resulted in an amino acid substitution located within a hypervariable region, whereas single nucleotide substitutions in both Vβ12 and Vα22 genes were found to be conservative. In contrast to the limited polymorphism observed with individual gene segments, TCR haplotypes are highly polymorphic. There is considerable polymorphism in the combination of markers inherited together in both TCRα and TCRβ haplotypes. In addition, variability in TCR gene complexes derives from the insertion or deletion of segments of DNA; two frequently occurring insertion/deletion related polymorphisms were found in the TCRβ complex. The locations of the IDRP were determined by the development of an extended map of the TCRβ complex showing that one involved a stretch of ~30 kb in the V region and another spans ~15kb near the C region. Analysis of 40 sibling pairs concordant for the relapsing-remitting form of Multiple Sclerosis (MS) revealed that a gene within the TCRβ complex or a closely linked locus influences susceptibility to MS.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00525-02 LIG

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Analysis of Human Killer T Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. O. Long	Visiting Scientist	LIG, NIAID
OTHERS:	M. Malnati	Visiting Fellow	LIG, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Immunogenetics

SECTION
Molecular Immunology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Inactive during current year.

PHS 6040 (Rev. 1/84) GPO 914-918

LABORATORY OF IMMUNOLOGY
1990 Annual Report
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PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Immunology
October 1, 1989 through September 30, 1990

Introduction

The Laboratory of Immunology is concerned with the elucidation of the fundamental mechanisms underlying immunologic responses. It has made rapid progress through the use of new technologies that have created a revolution in immunologic science. These are the techniques of modern molecular biology, monoclonal antibodies, long term lines of cloned normal and transformed lymphocytes, and flow cytometric analysis. These methods combined with newer techniques in protein chemistry and cell biology should allow the solution of many of the major problems that have concerned immunologists and should lead to important advances in efforts to regulate the normal and the disordered immune response.

The Transmembrane Region of IgM is Not Sufficient to Transduce a B Cell Activation Signal

Cross-linkage of membrane immunoglobulin (Ig) by antigens that express multiple copies of the same epitope or by anti-immunoglobulin antibodies results in the generation of a series of biochemical signals within the cell. These include increased inositol phospholipid metabolism, elevation of intracellular free calcium concentration, and the phosphorylation of a series of substrates at both serine and threonine residues and at tyrosine residues. The means through which cross-linkage of membrane Ig induces such signals has been enigmatic since IgM and IgD, the principal membrane isotypes, lack an appreciable cytosolic domain. However, the transmembrane domains of these Igs show a high degree of homology, both when compared across class or across evolutionary variants. This suggested that the transmembrane region might be essential or possibly sufficient for signal transmission. To test the latter idea, Laboratory of Immunology scientists prepared genetic constructs that encoded a chimeric membrane protein consisting of the external domains ($\alpha1$, $\alpha2$ and $\alpha3$) of the class I major histocompatibility complex molecule K^k and the transmembrane and cytosolic domains of the IgM heavy chain. The resulting protein ($K^k\mu$) was expressed in B lymphoma cells by transfection and transgenic mice expressing this protein as a cell surface "receptor" were developed. The capacity of this chimera, that could be cross-linked by antibodies to K^k epitopes and which bore the transmembrane regions of the IgM heavy chain, to transmit signals was examined. Cross-linkage failed to initiate increases in inositol phosphates or to elevate intracellular free calcium concentrations. Moreover, cross-linkage of $K^k\mu$ on both transfected B lymphoma cells and on transgenic B cells did not have any detectable biologic effect as measured by growth inhibition for the lymphomas or growth stimulation, in the presence of IL-4, for the transgenic B cells. These results thus indicate that the cross-linkage of the transmembrane region of IgM is not a sufficient stimulus for the generation of activation signals in B cells. (Tsang, Ishida, Chused and Paul, LI/NIAID; Mizuguchi, National Institute of Health, Tokyo).

Stimulation of T Cells by Anti-CD2 Antibody Does Not Require the Expression of the T Cell Receptor/CD3 Complex

T cells express a series of cell surface determinants capable of participating in the cell

activation process. In most cases, these molecules can only transmit biochemical signals into the cell and stimulate the production of interleukin-2 (IL-2) if the T cell expresses the T cell receptor/CD3 complex. On the other hand, the T cell membrane antigen CD2 is known to be expressed on thymocytes prior to their expression of T cell receptors. Since data exists that show that CD2 can stimulate T cell responses, it was essential to determine whether responses through CD2 required the expression of CD3. Obviously, if CD2 signalling requires the co-expression of CD3, it cannot play a role in responses of T cells prior to expression of CD3. To examine this point, a mouse T cell line was derived that failed to express the T cell receptor/CD3 complex due to the deletion of all functionally rearranged T cell receptor α and β genes. The human CD2 gene was transfected into this line and transfectants expressing high levels of human CD2 were isolated. CD2 cross-linking of such cells resulted in modest but significant IL-2 production, firmly establishing that CD3 expression is not required for CD2 mediated T cell activation. Thus, this cell surface molecule may have a major role in T cell activation independent of the T cell receptor/CD3 complex. (Saito and Germain, LI/NIAID).

Cloning of Murine Vitronectin Receptor and Demonstration of its Role in the Activation of a Dendritic Epidermal T Cell Line

Integrins are a large family of cell surface molecules many of which are receptors for extracellular matrix proteins. Laboratory of Immunology scientists have identified a novel member of the integrin family that is expressed on many T cells that express receptors of the γ/δ type. This integrin was originally identified thorough the use of a monoclonal antibody that recognized an antigen expressed on the surface of many dendritic epidermal T cells of the γ/δ type. Molecular cloning of this antigen using an expression system demonstrated that it was an integrin that had not been previously defined in the mouse. It was then shown that this integrin mediated binding to fibronectin, vitronectin and fibrinogen and that this binding could be inhibited by the tetrapeptide, RGDS. Based on its specificity and pattern of inhibition by antipeptide antibodies it was concluded that the molecule was the murine vitronectin receptor. This represent the first definitive demonstration that vitronectin receptors are expressed on lymphoid cells. It was further demonstrated that the vitronectin receptor played a role in lymphokine production by a population of dendritic epidermal cells stimulated at the same through their T cell receptors. These cells (T195/BW) produced interleukin-4 when the cells were cultured in the presence of a source of extracellular matrix proteins that express the RGDS sequence. More strikingly, a synthetic peptide containing RGDS coupled to a series of highly charged amino acids allowed IL-4 production. The population of T cells represented by the dendritic epidermal T cells used in these experiments all express the $V\gamma4$, $V\delta6$ receptors. Such cells are found in high frequency in the thymus at birth. The vitronectin receptor may play a critical role in the development and function of this population of γ/δ cells and perhaps more widely throughout the T cell system. Additional studies are aimed at understanding how the vitronectin receptor regulates IL-4 production by these T cells and at determining the physiologic importance of the vitronectin receptor in lymphocyte function. (Yokoyama, Maxfield, Roberts and Shevach, LI/NIAID; Cohen, LIR/NIAID; Koning and Coligan, BRB/NIAID).

A Deletion in an Immunoglobulin J_H-Proximal V_H Gene Profoundly Affects Expression of Cis-V_H Genes in Rabbits

Immunoglobulin (Ig) heavy chain variable region (V_H) genes are arrayed tandemly 5' of the genes that encode the D and J elements and the constant region genes. This constellation of

genes constitutes the complex Igh locus. The mechanisms that govern the utilization of distinct V_H genes are poorly understood. Recent studies with a mutant rabbit (*Alicia*) have provided substantial insight into this process. Rabbits are unique among animals whose Ig genes have been studied in that allelic allotypic determinants (a1, a2 or a3) exist on many serum Ig molecules. These are located within the portion of the Ig encoded by the V_H element. The basis of the allelic behavior of V_H allotypes has been enigmatic since the Igh locus has a large number of V_H genes distributed over a substantial genetic distance. The means through which this genetic arrangement could be compatible with simple Mendelian inheritance of the allotypic markers has been the subject of considerable interest. The *Alicia* rabbit bears a mutation in the Igh locus (*ali*) which results in marked depression of the expression of the a2 allotype, the allotypic determinant encoded by the haplotype in which the *ali* mutation occurred. Genomic analysis of the Igh loci of normal and *ali* rabbits with infrequently cutting restriction enzymes and transverse alternating field electrophoresis reveals a restriction fragment in *ali* that is 10-15 kb smaller than the comparable region in normal rabbits. Hybridization analysis indicates that this represents a deletion that includes a segment including the most J_H-proximal V_H genes. This suggests that the 3' V_H genes play a profound role in the regulation of Ig gene expression from that chromosome. These findings may have great significance in understanding the means through which the B cell repertoire is formed. (Allegrucci, Newman and Mage, LI/NIAID; Meier and Kelus, Basel Institute of Immunology).

Identification of Residues in Class II Sites to Which a Cytochrome C-Peptide Binds

T cell receptors recognize a complex that consists of peptides derived from antigens, by intracellular proteolytic digestion, bound to a specialized groove in class I or class II major histocompatibility complex (MHC) molecules. In order to examine the structural basis of this binding, LI scientists have utilized a series of mutants of class II molecules and tested the capacity of an immunogenic cytochrome c peptide to bind to the resultant sites. The binding experiments involved the use of a biotinylated cytochrome c peptide and the direct measurement of the binding of the labelled peptide, using stretavidin. The analysis of binding to $\alpha\beta$ dimers that are derived from mutant $E\beta$ chains, derived by site-directed mutagenesis, paired to wild type $E\alpha$ chains indicate that polymorphic residues at positions 29, which is in the β sheet constituting the floor of the site, and positions 72 and 75, which are in the α helical walls were critical elements. When compared to the crystal structure of class I MHC molecules, these result suggests that the binding site of class II molecules consists of a set of local binding subsites. This work promises to have great importance in understanding the structural determination of immunogenecity and may make possible the design of peptides that could interfere in the process of antigen recognition. (Ronchese, Racioppi and Germain, LI/NIAID).

β_2-Microglobulin is Required for Efficient "Loading" of Peptides into A Class I MHC Molecule

T cell receptors recognize a complex consisting of a peptide derived from an antigen and a class I or class II MHC protein. Recent crystallographic analyses of class I molecules indicates the existence of a groove formed by the $\alpha1$ and $\alpha2$ domains of the class I molecule that forms the peptide-binding site. A critical question has been how peptide is loaded into this site. This process is believed to occur normally intracellularly in the course of the processing and assembly of the class I MHC molecule. In order to study this process in a more direct way, Laboratory of Immunology scientists have prepared a soluble form of the class I molecule

H-2D^d, using genetic engineering to place the $\alpha 1$, $\alpha 2$, and $\alpha 3$ domains on the carboxy terminal region of the non-polymorphic class I-like molecule Q10, which is normally secreted. This soluble D^d molecule can be purified and when loaded with a peptide derived from the HIV virus (peptide 18 [p18]) can be used to stimulate a hybridoma specific for peptide 18 presented by H-2D^d. This thus provides an assay for the successful loading of p18 into D^d. When purified D^d is exposed to p18, it was observed that loading is dependent upon the presence of serum. The serum component responsible for this effect was demonstrated to be β_2-microglobulin. These studies strongly suggest that either the binding of peptide to class I molecules requires the presence of β2-microglobulin or, more likely, that the peptide/class I chain complex is unstable in the absence of β2-microglobulin. It has previously been reported that without peptide, the class/β2-microglobulin complex is unstable. These results thus suggest that only the ternary complex consisting of the class I polypeptide, the antigen-derived peptide and β2-microglobulin is stable and, most likely, that this is the only assemblage that can stimulate T cell responses. This observation also has great experimental implications since it suggests ways in which the binding of peptides to class I molecules can be directly studied in vitro. (Kozlowski and Margulies, LI/NIAID).

Efficiency of Expression of Class II MHC Molecules is Controlled at the Level of Intracellular Transport

Class II major histocompatibility complex (MHC) molecules consist of α and β chains that associate with one another specifically and jointly create a site to which antigenic peptides can bind. Laboratory of Immunology scientists had observed that there was a specificity in the interaction of distinct polymorphic forms of α and β chains. Thus, some combinations of α and β chains were efficiently expressed on the cell surface while other forms were poorly expressed. During the last year, LI scientists have shown that the variability in the efficiency of expression of distinct αβ dimers is not determined by a failure of the dimers to form, but rather because these dimers are not efficiently transported from the rough endoplasmic reticulum. Moreover, this process is controlled by the binding of invariant chain (Ii) to the dimer. Thus, in invariant chain deficient cells, αβ combinations that are normally moderately well expressed show much diminished density on the cell surface which can be greatly enhanced by the co-expression of Ii. This indicates a critical need for Ii in regulation of intracellular transport of class II molecules and suggests that the capacity of Ii to bind to αβ dimers is key to the level of expression of such dimers. Parallel studies suggest that αβ dimers that have successfully been transported into a post-Golgi compartment adopt a denaturation resistant conformation whereas molecules that have not completed this transit and are still associated with Ii have low resistance to denaturation. This process of transition from a denaturation sensitive to a denaturation resistant conformation is enhanced by the presence of antigen, suggesting that the binding of an antigen-derived peptide into the site of the αβ dimer results in a conformational change in the αβ dimer. (Sant, Layet, Hendrix and Germain, LI/NIAID)

Regulation of IL-2 Gene Expression by DNA-Binding Factors for κB and Related Sequences

Gene expression is controlled by the action of a series of regulatory proteins that bind to defined sites in the promoter regions of the specific genes. NF-κB is one such regulatory protein that is involved in the control of expression of a large variety genes; it recognizes a sequence designated κB. Since the κB site and κB-like sites are found in the promoter regions of multiple genes, it is obvious that regulatory mechanisms must exist to allow the specific

activation of individual genes from the large group of genes with promoters containing κB sequences. Laboratory of Immunology scientists have undertaken an examination of the regulation of expression of the genes for interleukin-2 (IL-2) and its receptor with particular emphasis on the role of κB and related sequences. They have observed that another DNA binding factor, NF-ILT, must bind to a site near κB to allow NF-κB to act on the IL-2 gene in T cells. Furthermore, a novel DNA-binding factor has been discovered, NF-CYT1, which directly interacts with the κB site in the IL-2 gene. This factor may block the action of NF-κB and thus has characteristics of a repressor protein. NF-CYT1 also binds to a site in the enhancer region of HIV and may serve to suppress its expression in T cells. These experiments promise to give insights into the mechanisms of gene regulatory events during lymphocyte activation. (Kuang, Kang and Lenardo, LI/NIAID).

Induction of IL-4-Producing T Cells In Vitro

Interleukin-4 is a major regulator of immune responses. It controls switching to the expression of IgE and in addition has a multiplicity of effects on B cells, T cells, macrophages, mast cells and other cells of the lymphohematopoietic system. The understanding of the regulation of its production is thus a goal of considerable importance. Utilizing a highly sensitive assay dependent upon a IL-4 selective cell line developed in the Laboratory of Immunology, it has been shown that T cells from naive donors are very poor producers of IL-4 in response to stimulation by cross-linkage of the CD3 complex. The frequency of IL-4-producing T cells is ~1/1000 and the production of IL-4 by these cells is dependent upon the presence of IL-2. Immunization of mice by injection with the polyclonal stimulant anti-IgD or by infection with *Nippostrongylus brasiliensis* causes a striking increase in the frequency of IL-4-producing cells, to ~1/50 to 1/200. These cells remain dependent upon IL-2 to produce IL-4. Indeed, it can be shown that stimulation with anti-CD3 antibodies and IL-2 for two hours results in striking induction of IL-4 mRNA while stimulation with anti-CD3 and anti-IL-2, to neutralize any endogenously produced IL-2, causes little if any IL-4 mRNA to appear. IL-4 producing T cells can be induced in vitro to develop from the naive T cell population by culture for 3-5 days with anti-CD3 and IL-2 and IL-4. Upon restimulation with anti-CD3 and IL-2, the frequency of IL-4-producing cells rises to ~1/20. Strikingly, both $CD4^+$ and $CD8^+$ T cells primed in this way produce IL-4; the frequency of IL-4-producing cells in the two populations is essentially the same. These studies thus demonstrate one means through which T cells can be induced to differentiate toward the production of IL-4. Efforts are now underway to determine the physiological significance of this pathway and to determine what other stimulants can also result in populations of small resting T cells acquiring the capacity to produce IL-4. (Seder, Ben-Sasson, LeGros, and Paul, LI/NIAID).

Spleen and Bone Marrow Cells Expressing High Affinity Fcε Receptors Produce IL-4

Interleukin-4 and a set of related lymphokines (IL-5, IL-3 and GM-CSF) are produced by a specialized sub-population of T lymphocytes and, as Laboratory of Immunology scientists have shown, by transformed mast cells and by growth factor-dependent mast cell lines. Studies conducted in the Laboratory of Immunology have now demonstrated that a normal population of cells exists in the spleen and bone marrow of naive mice that can produce IL-4 in response to cross-linkage of high affinity Fcε receptors, presumably FcεRI. IL-3 strikingly enhances IL-4 production by such cells in response to FcεR cross-linkage. Infection of mice with the nematode parasite *Nippostrongylus brasiliensis* or injection of anti-IgD antibodies strikingly increases

the IL-4 producing capacity of the FcεR+ population of spleen and bone marrow cells. This cell population has been purified from spleen and bone marrow by initial depletion of cells bearing B and T lymphocyte markers and subsequent cell sorting based on the binding of IgE to the high affinity FcεR. The purified cells constitute ~0.1% of spleen cells and 1% of bone marrow cells. In animals that have been stimulated with polyclonal activators that stimulate IL-4-dependent increases in serum IgE, the frequency among bone marrow cells can rise to 5-10%. Utilizing a recently developed assay allowing the measurement of single IL-4-producing cells, it was estimated that ~1/3 of the purified cells can produce IL-4 in response to cross-linkage of FceR. This cell population also contains mast cell precursors, providing direct evidence that many mast cell precursors already express high affinity FceR. These studies promise to give insight into the contribution of mast cell-derived lymphokines to pathophysiological states and in protective immunity. (Seder, Ben-Sasson, Plaut, LeGros and Paul, LI/NIAID).

Mice Expressing the Soluble D^d Class I Major Histocompatibility Complex Molecule as a Transgene Show Partial Tolerance to D^d

The construction of genes for soluble class I MHC molecules has enabled Laboratory of Immunology scientists to determine whether membrane expression of class I MHC molecules is essential to their establishment of a state of immunological tolerance. To that end, transgenic C57BL/6 mice expressing the transgene for soluble D^d were examined for their capacity to immunologically respond to D^d. The transgenic mice were shown to express soluble class I protein in their serum at a steady state level of about 100 μg/ml. They expressed no cell surface H-2D^d. Using a sensitive and specific RNAse protection assay, it was shown that mRNA for the soluble D^d molecule was expressed in a wide variety of tissues that normally express H-2D^d including lymphoid organs (spleen, lymph node, thymus) as well as heart, skeletal muscle, kidney, liver, at levels comparable to those of the endogenous H-2 genes. The capacity of the transgenic mice to make immune responses to D^d was tested by immunizing them with cells or skin from a C57BL/6 mouse expressing a transgene for the membrane form of D^d. The recipients mounted a normal cytotoxic T cell response specific for D^d, failed to make anti-D^d alloantibodies and rejected skin from the D^d donor, although more slowly than the parental (i.e. non-transgenic) C57BL/6 mouse. These results suggest that a state of partial tolerance to D^d can be initiated by the soluble D^d molecule. The most likely explanation is that CD4+ T cells specific for D^d have been tolerized resulting in the failure of antibody production and the slower graft rejection. CD8+ T cells on the other hand are not tolerized, explaining the normal generation of a cytotoxic T cell response. These results thus suggest that the requirements for tolerance induction by distinct forms of T cells may be quite different and that cell surface but not soluble class I MHC molecules are potent inducers of tolerance for cytotoxic T cell responses. (Hunziker and Margulies, LI/NIAID).

Isolation of cDNA for Murine Ly-49 and its Relation to NK Cell Function

Laboratory of Immunology scientists had prepared a monoclonal antibody (A1) that recognized a disulfide linked homodimer with 44 kD subunits expressed on the surface of rare T cell tumors. Two color immunofluorescence demonstrated that this molecule was expressed exclusively on the surface of a portion of freshly isolated cells that express NK1.1, strongly suggesting that the antibody recognized a subpopulation of NK cells. Molecular cloning of the cDNA for the antigen recognized by A1 revealed that the encoded protein was a type II integral

membrane protein that is a member of the supergene family that includes the human and mouse low affinity receptors for IgE (CD23) and the asialyglycoprotein receptor. The molecule has been designated Ly-49. Through the use of restriction fragment length polymorphisms in inbred and recombinant inbred mouse strains, Ly-49 has been mapped to the distal portion of chromosome 6. This places it close to the gene for NK1.1, a most provocative finding in view of the expression of both these molecules as cell surface antigens on NK cells. The finding that Ly-49 is found on only some NK1.1-bearing cells suggests that the antibody may sub-divide NK cells and also raises the question of the structural and functional relationship between Ly-49 and NK1.1 and of the role of this molecule in NK cell function. (Yokoyama and Shevach, LI/NIAID).

<u>Extracellular ATP May Mediate Killing by Cytotoxic T Cells</u>

Recent evidence that exocytosis is not required for target cell killing by cytotoxic T cells (CTLs) strongly implies that a mechanism other than secretion of granule contents, such as perforin, can account for killing. LI scientists have proposed that extracellular ATP (ATP_o), produced by the CTL provides such a killing mechanism. This proposal was based on the initial findings that ATP_o can kill a variety of target cells and that CTL are relatively resistant to ATP_o-mediated killing. Based on this, further evidence was sought to implicate ATP_o as a killing mediator. It was demonstrated that activation of CTL lines by T cell receptor cross-linkage or by concanavalin A (Con A) causes a striking increase in ATP_o and that ATP-degrading enzymes block killing of target cells by CTL but do not affect T cell receptor-mediated transmembrane signalling in CTL. Both CTL and target cells express ecto-protein kinases that utilize ATP_o to phosphorylate cell surface proteins. Finally, induction of ATP_o increase as a result of T cell activation is independent of need for extracellular calcium. The latter is particularly important since CTL can lyse target cells in the absence of extracellular calcium but secretion of CTL granule contents is calcium dependent. These results strongly suggest that ATP_o is involved in the cytotoxic mechanism and suggest that it may be the principal means through which CTL kill their targets. (Filippini, Taffs, Agui and Sitkovsky, LI/NIAID).

<u>Synthetic Peptide Substrates, Pseudosubstrates and Inhibitors of Protein Kinases Block Cytotoxic T Cell Effector Functions</u>

Laboratory of Immunology scientists have proposed that the functions of intracellular kinases may be inhibited by incubating unpermeabilized cells with synthetic peptide substrates, pseudosubstrates or inhibitors of protein kinases. Using cloned cytotoxic T cells (CTLs) to test this idea, it was observed that incubation of these cells with a synthetic peptide substrate and an inhibitor of cGMP-dependent protein kinase, a synthetic substrate of smooth muscle myosin light chain kinase, and a peptide corresponding to the tyrosine phosphorylation site in $pp60^{src}$ strongly inhibited both T cell-receptor and IL-2 mediated CTL growth as well as a series of other induced functions of these cells. By contrast, a peptide substrate for multifunctional calmodulin-dependent protein kinase, a substrate for a tyrosine-specific protein kinase, a substrate for a cAMP-dependent protein kinase, and kemptide had no inhibitory function. It was directly shown that the capacity of the synthetic peptides to block cytotoxic activity was due to an action on the CTL and not the target cell. The capacity of added synthetic peptides to block intracellular events could be explained if the peptides gain access to the cell and can partition into the compartments in which the kinases normally medidate their function. Consistent with

this, minor changes in the structure of the peptides profoundly alters the inhibitory activity of the peptide. It is proposed that certain inhibitory peptides can gain access to the cell and profoundly inhibit kinase functions. It is possible that such an approach may have great value in the manipulation of immune responses. (Taffs and Sitkovsky, LI/NIAID).

Induction of Autoimmune Disease in Progeny by Treatment of Pregnant Mice with Cyclosporine A

Cyclosporine A (CsA), although a potent suppressor of T cell-dependent immune responses, has been reported to induce autoimmunity in mice after treatment is stopped. Laboratory of Immunology scientists have been interested in the role CsA administration to pregnant mice might play in autoimmunity development in their offspring. To that end, pregnant mice were treated for a two week period with CsA using a mini-osmotic pump. The offspring had lymphoid systems that were grossly normal, with the exception of some reduction in the percentage of CD4+ T cells in the neonate. Between the ages of 2 and 6 months, some of these animals developed elevated titers of antibodies to gastric mucosal antigens, as measured by an ELISA assay. In the limited numbers of animals that have been sacrificed, evidence of gastritis has been observed histologically. No evidence for expression of normally deleted Vβ gene segments in the CsA-treated mice has been observed, suggesting that escape from "deletional tolerance" may not explain CsA-induced autoimmunity. An alternative possibility is that CsA may inhibit the development or function of a population of regulatory T cells that normally limit the action of autoreactive T cells. These results suggest that great care should be exercised in CsA treatment regimens that may involve exposure of fetuses to this drug. (Classen and Shevach, LI/NIAID).

Laboratory of Immunology scientists play important roles in the U.S. and international immunological communities. They serve on editorial boards of many scholarly publications. Dr. Ethan Shevach is editor-in-chief of the Journal of Immunology, and is a member of the editorial boards of Cellular Immunology, of the Journal of Immunological Methods and of Current Protocols in Immunology and is a member of the Council of Biology Editors.

Dr. William Paul is the editor of the Annual Review of Immunology. He is an advisory editor of the Journal of Experimental Medicine, an associate editor of Cell, and a member of the editorial boards of Immunological Reviews, of the Journal of Molecular and Cellular Immunology, of Cell Regulation and of Cytokine. He is a transmitting editor of International Immunology and a corresponding editor of the Proceedings of the Royal Society, Series B. He is a member of the editorial advisory board of Advances in the Regulation of Cell Growth. He edited a volume of "Readings from the Scientific American" entitled Immunology: Recognition and Regulation.

Dr. Ronald Germain is a deputy editor of the Journal of Immunology and is a member of the advisory editorial board of the Journal of Molecular and Cellular Immunology and of the Annales de l'Institut Pasteur. Dr. Rose Mage is a member of the editorial board of Immunogenetics. Dr. Michael Lenardo is an associate editor of Molecular and Cellular Biology. Dr. John Inman is a member of the editorial board of Analytical Biochemistry and is an advisory editor of Molecular Immunology. Dr. Thomas Chused is a member of the editorial board of Cytometry.

Dr. Paul is a member of the committee to visit the Division of Medical Sciences of the Board of Overseers of Harvard College; of the advisory committee of the Pew Scholars Program in the Medical Sciences; and of the selection committee for the RJR/Nabisco Scholars Award in Immunology. He is chairman of the board of scientific consultants of the Memorial-Sloan Kettering Cancer Center and of the advisory committee of the Harold C. Simmons Arthritis Research Center. He is a member of the scientific review board of the Howard Hughes Medical Institute, of the Scientific Advisory Committee of the Cancer Research Institute, Inc., and of the Research Visiting Committee of the State University of New York Health Science Center at Brooklyn. He was a member of the steering committee of the NIAID Task Force on Immunology and Allergy. He completed terms as a member of the board of scientific advisors of the Jane Coffin Childs Fund for Medical Research and as a member of the board of directors of the Foundation for Advanced Education in the Sciences.

Dr. Paul was elected to membership in the Institute of Medicine of the National Academy of Sciences, U.S.A. and has accepted the position of Secretary-General of the Ninth International Congress of Immunology, to be held in San Francisco in 1995. He serves as a member of the scientific organizing committee of the International Conferences on Lymphocyte Activation and Immune Regulation. Dr. Paul organized the Jane Coffin Childs Fund Symposium of Tolerance and Autoimmunity. He presented the "Distinguished Alumnus Lecture" at University Hospital, Boston, was the 1990 Cancer Immunology Visiting Professor at the University of Texas, Southwestern Medical Center and delivered the Anderson Lectures at the University of Virginia. He was an invited lecturer at the symposium held at the annual meeting of the Institute of Medicine, at the annual Frederick Stohlman, Jr. Memorial Symposium, at the annual fellows

symposium of the Life Sciences Research Foundation, at the Symposium on T Cells and Cytokines in Health and Disease at Airlee House, at the First International Congress on Cytokines: Basic Principles and Clinical Applications in Florence, Italy, at the International Conference on Cellular Mechanisms in Malaria Immunity in Bethesda, at the First International Congress on Inflammation in Barcelona, Spain and at the American Association of Immunologists Advanced Immunology Course in St. Louis.

Dr. Shevach is a member of the Scientific Advisory Committee of the American Leprosy Foundation. He was an invited speaker at the symposium on The Functional Role of Antigen Presenting Cells, Baden, Austria, at the symposium on Development and Function of $\gamma\delta$ T Cells, Segovia, Spain and at the Gordon Conference on Immunobiology and Immunochemistry, Ventura, CA.

Dr. Germain was a session chairman at the International Conference on Cellular Mechanisms in Malaria Immunity in Bethesda, was an invited speaker at the Gordon Conference on Immunochemistry and Immunobiology, at the 7th HLA-H2 Workshop in Elmau, FRG, and at the plenary session on "Molecular Strategies for MHC Molecules" at the annual meeting of the American Society for Biochemistry and Molecular Biology/ American Association of Immunologists Annual Meeting.

Dr. Mage serves as the American Association of Immunologists' representative to the board of the American Type Culture Collection (ATCC) where she is a member of its Executive Committee. She is also a member of the Board of Directors of the Foundation for Advanced Education in the Sciences and a Fellow of the American Association for the Advancement of Science.

Dr. David Margulies was an invited speaker at the Jackson Laboratory Meeting on "Transgenics and Mutants in MHC Research". He is a member of the Immunology and Immunotherapy Advisory Committee of the American Cancer Society and of the Fellowship Review Committee of the Arthritis Foundation.

Dr. Michail Sitkovsky was co-chairman of sessions at the International Cell Mediated Cytotoxicity Workshop and of a symposium at the annual meeting of the American Association of Immunologists.

Dr. Michael Lenardo received an Investigator Award in Immunology from the Cancer Research Institute, Inc. He was an invited speaker at the Winter Immunology Conference at Asilomar, CA and at the annual meeting of the Mid-West Conference of Immunologists. He was an invited speaker at the conference on Molecular Factors of Stem Cell Growth in Moscow, USSR, at the Shemyakin Institute for Bioorganic Chemistry in Moscow and at the meeting on "Modern Trends in Human Leukemia" in Wilsede, FRG. He serves as an advisor in molecular biology for the NIH Immunological Sciences Study Section.

In addition, Laboratory of Immunology scientists presented seminars at major universities, medical schools and research institutes in the United States and abroad.

Administrative, Organizational and Other Changes

The Laboratory of Immunology continues to be a major training center for young immunologists. During the past year several individuals completed post-doctoral training or sabbatical periods in the Laboratory of Immunology. Among these were Ulus Atasoy, Yehoshua Gozes, Nichola Hole, Peter Hornbeck, Barbara Newman and Antonio Procopio. Each of these scientists made important contributions to the Laboratory of Immunology research program. It is anticipated that they will have very productive research careers.

During the past year, several scientists joined the Laboratory of Immunology for post-doctoral training or sabbatical visits. They included Sergey Apasov, Clara Brando, Hsiao-Kun Chu, Dan Eilat, Patrizia Fuschioti, Mariagrazia Grilli, Sang Kang, Kwang Ho Lee, Felicity Lynch, Frank Redegeld, Scheherazade Sadegh-Nasseri, Daniel Salamon, Peter Weinstein and Zheng-Sheng Ye. It is expected that they will continue a tradition of excellence established by a long a series of outstanding trainees in the Laboratory.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00030-22 LI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antigen Recognition and Activation of Immunocompetent Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William E. Paul	Chief	LI, NIAID
Others:	A. Keegan	NRC Fellow	LI, NIAID
	J.-L. Boulay	Visiting Fellow	LI, NIAID
	T. Tanaka	Visiting Fellow	LI, NIAID
	Z. S. Ye	IRTA Fellow	LI, NIAID
	M. Plaut	Guest Researcher	LI, NIAID

COOPERATING UNITS *(if any)*

NCI (J. Pierce); NIAID (J.-P. Kinet)

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	1.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lymphokine production by cells of the immune system can be initiated by cross-linkage of cell surface receptors. This is particularly striking for T cells where the relevant receptors are the α/β or γ/δ T cell receptors, for mast cells where the receptors involved are FcεRI and FcγRII, and for NK cells where the receptor involved is FcγRIII. The production of lymphokines mediated in this way is strikingly modulated by the presence of lymphokines; interleukin-4 (IL-4) production by naive T cells is strikingly enhanced by IL-2 while IL-4 production by cells of the mast cell lineage has a requirement for IL-3. Mast cell production of IL-4 and of other lymphokines, such as IL-3, depends upon cross-linkage of Fc receptors. For lymphokine production in response to FcεRI cross-linkage, there are several very interesting features. Lymphokine secretion can be detected within ~1 hr of stimulation, requires continued presence of the cross-linking stimulus and is inhibited by excessive cross-linkage. IL-3 and IL-4 production by these cells is markedly enhanced by pretreatment of the cells with high concentrations of IL-3. Low concentrations of IL-3 or IL-4, although they cause the cells to grow, do not prepare them to produce IL-3. Similar effects have been observed in normal cells; IL-4 production by FcεRI+ cells in response to receptor cross-linkage is strikingly enhanced by pretreatment with IL-3.

The functions of IL-4 are mediated by binding to a high affinity receptor. The major molecular species that is cross-linked to membrane-bound ^{125}I-IL-4 is a 70,000 dalton molecule (p70) while the major IL-4-binding molecules purified from cell lysates or from membrane-labelled cells have weights of 120,000 (p120) and of 40,000 (p40). The former is the major form of the cell surface receptor; the latter appears to be a secreted form of the receptor. The nature of p70 has been enigmatic. We have shown that p70 has a peptide map, for ^{125}I-IL-4-bound peptides, similar to p120, implying that it is a fragment of p120 but the conditions under which this fragment is generated and its physiologic significance remain to be established.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00035-15 LI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Specificity in Immune Responses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	John K. Inman	Section Head	LI,NIAID
Others:	Andrew Lees	NIH Special Volunteer Dept. of Medicine	USUHS
	Patricia F. Highet	Technician	LI,NIAID

COOPERATING UNITS (If any)

Dept Medicine, USUHS, Bethesda MD (Drs. F. D. Finkelman, J. J. Mond); Dept Biophysics, Johns Hopkins University School of Medicine, Baltimore, MD (Prof. H. M. Dintzis).

LAB/BRANCH

Laboratory of Immunology

SECTION

Bioorganic Chemistry Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8 (1.0 not paid through NIH)	2.0	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A major part of this project is directed toward the design and synthesis of highly specific and efficient immunomodulators. Effector molecules (e.g., monoclonal antibodies directed against designated cell surface proteins) are multiply and covalently linked to soluble polymer carriers of high molecular weight using heterobifunctional reagents. In some studies, protein-protein heteroconjugates have been prepared. New cross-linking reagents have been designed, synthesized and used as part of this project. Special emphasis has been given to improving the chemistry for functionalizing antibody molecules through their polysaccharide moieties and to the cross-linking of these derivatives to functionalized carriers. Collaborative studies employing the immunomodulators have dealt largely with a variety of cell surface factors involved in activation, growth and differentiation of B lymphocytes and presentation of antigen to T cells. For example, it has been shown (Finkelman, Mond et al.) that anti-IgD alone delivers a weak mitogenic signal to resting B cells, but when it is multiply linked to a soluble polymer such as dextran of MW ≥ 200,000, strong mitogenic stimulation occurs at antibody concentrations ranging from 0.01 to 1.0 ng/ml. These conditions of stimulation are believed to closely approximate normal, T cell-independent antigenic signaling that appears to involve different pathways of transduction than believed to predominate for non-cross-linked signals at higher concentrations. Another objective of this project is to explore the property of general multispecificity of antibodies and other receptors. A study has been completed that measured the distribution of binding constants of a monoclonal antibody for a large collection of diverse compounds (not related to the homologous immunizing hapten). The results strongly support the statistical basis for multispecificity. Quantitative affinity chromatography was used to measure binding constants down to very small values. Techniques for preparing specialized affinity adsorbents were developed for these and other studies. Further work is planned for exploring alternative modes of complementation of ligands in receptor sites and relating these findings to specificity in immune systems and networks.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00036-25 LI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ig Genetics: Ontogeny and Differentiation of Cells of the Rabbit Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. G. Mage	Section Head	LI, NIAID
Others:	B. Newman	IRTA Fellow	LI, NIAID
	N. Hole (to 7/90)	Visiting Fellow/Associate	LI, NIAID
	M. Allegrucci (to 5/90)	Visiting Associate	LI, NIAID
	P. Fuschiotti (from 5/90)	Visiting Associate	LI, NIAID

COOPERATING UNITS (if any)

LOM, NIDR (N. Harindranath); Dept. Microbiol., Loyola Stritch Sch. Med. Chicago, (K. Knight); Dept. Biochem., Univ. Geneva, Switzerland (J. -C. Jaton); Natl. Univ. Mexico, Mexico City, Mexico (E. Lamoyi).

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Immunogenetics Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.88	1.88	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We use techniques of classical immunogenetics and of molecular biology to study the genetics of rabbit immunoglobulins (Igs) and T cell receptors (Tcr) and to investigate the regulated expression of Ig and Tcr genes during lymphoid cell development. We have reported that there are evolutionarily conserved IgH enhancer sequences and a donor splice site in the intron between the Ig heavy chain J-region and the IgM heavy chain constant region ($C\mu$) genes. The presence of the conserved splice site sequences in the JH-$C\mu$ intron region of the human, mouse and rabbit genomes and the utilization of the splice sites to process sterile $C\mu$ mRNA transcripts expressed by developing B cells of mice and rabbits suggests that these transcripts may play a regulatory role during B-cell development.

Some rabbits are unusual in having three different copies of Tcr $C\beta$ genes. The third gene is a chimeric $C\beta2$-$C\beta1$ genomic Tcr β chain gene that may have arisen by an unequal crossing over event analogous to that which may have deleted $C\beta1$, $D\beta2$ and $J\beta2$ in NZW mice. We demonstrated this in Southern analyses of both total genomic DNA and two different genomic clones of ~6 and ~14 kb as well as by sequencing cloned genomic DNA.

Rabbits were bred at the NIH to produce elevated levels of lambda light chains lacking c21 and expressing only c7. These rabbits were shown to produce mRNA and proteins with sequences corresponding to the products of a previously identified genomic lambda light chain gene, $C\lambda6$. The production of c21 is known to be due to expression of $C\lambda5$. The c21-negative phenotype reflects deletion of a region including $J\lambda5$. The c7-negative phenotype also appears to result from a deletion.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00134-28 LI

PERIOD COVERED

October 1 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Control of Immunoglobulin Synthesis in Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Richard Asofsky, Section Chief, LI, NIAID

Others: J. Little, IRTA Fellow, LI, NIAID; S.-I. Minato, Visiting Fellow, LI, NIAID; H.-K. Chu, Visiting Associate, LI, NIAID; F. Zhao, Visiting Associate, LI, NIAID; A. E. Brooks, Biological Technician, LI, NIAID; J. A. Brooks, Biologist, LI, NIAID; T. M. Cox, Co-Op Student, LI, NIAID

COOPERATING UNITS (if any)

W. McCabe & C. Sulis (Infectious Diseases Unit, Boston City Hospital); J. J. Mond (Dept of Medicine, USUHS Med. Sch); J. Abrams (Dept of Immunology, DNAX Research Institute)

LAB/BRANCH

Laboratory of Immunology

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	5.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Certain B lymphoma and B hybrid cell lines can be induced to differentiate by anti immunoglobulin or LPS. LPS containing bacterial peptides is more effective than phenol-prepared LPS (Phe-LPS) in inducing terminal growth, but in contrast to Phe-LPS is a poor inducer of Ig secretion. Fetal calf serum contains factors which in sufficient amounts blocks this inductive effect. Medium conditioned by growth of hybrid TH 2.2 has a similar effect. Cytokines and lymphokines have pleiotropic effects on the growth and differentiation of different cell lines; e.g. GM-CSF (perhaps an autocrine factor) has no effect on growth of line TH 2.2, but inhibits line F6.14.7. GM-CSF further inhibits TH 2.2 cells induced with LPS, but enhances the growth of F6.14.7 cells so induced. IL-4 has no effect on TH 2.2. It inhibits growth of uninduced F6.14.7, greatly enhances growth of this line induced with LPS, but has little effect on the line induced with DXS.

Small purified murine B cells from spleen are induced by anti IgD and anti IgM coupled to Sepharose beads or high molecular weight dextran to secrete GM-CSF and a small amount of IL-3. The lymphokine activities were assayed on responsive cell lines, and each was specifically blocked by an appropriate monoclonal antibody.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00224-09 LI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Monoclonal Antibodies as Probes for T Cell Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. M. Shevach	Section Head	LI, NIAID
Others:	B. Classen	Staff Fellow	LI, NIAID
	K. Roberts	Visiting Associate	LI, NIAID
	D. Wilde	Med Staff Fellow	LI, NIAID
	G. Dos Reis	Visiting Scientist	LI, NIAID
	F. Lynch	Visiting Fellow	LI, NIAID
	C. Brando	Visiting Associate	LI, NIAID

COOPERATING UNITS (if any)

Biological Resources Branch, NIAID, NIH (J. Coligan)

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9.0	7.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major objectives of our studies are to characterize cell surface structures on T lymphocytes and on accessory cells which play critical roles both in vivo and in vitro in the process of lymphocyte activation :

1) We have developed monoclonal antibodies (mAb) to a T lymphocyte activation antigen and demonstrated in biochemical studies that it is the vitronectin receptor (VNR) which is capable of interacting with the extracellular matrix (ECM) proteins -vitronectin, fibronectin, and fibrinogen. The VNR appears to play a critical role as an accessory molecule for a subpopulation of T cells which express the $\gamma\delta$ T cell receptor (TCR). $\gamma\delta$ T cell lines and hybridomas which express the $C\gamma4$, $V\delta6$ chains appear to recognize an autoantigen; however, activation of these lines requires that, in addition, to engagement of the TCR by antigen, the VNR must also bind to its ligand on ECM-proteins.

2) We cloned a cDNA for a cell surface disulfide linked 44 kD dimer (Ly-49) which is a member of a large multigene family and which is expressed on rare T cell tumors. We have mapped the gene which encodes this antigen to the short arm of mouse chromosome 6 near the gene which encodes the murine natural killer (NK) cell marker, NK1.1. Ly-49 also may play a role in NK cell function because 30% of highly purified NK cells express Ly-49; it is also possible that NK1.1 is a member of the Ly-49 multigene family.

3) In continuing studies of cell surface antigens which are coupled to the membrane via a phosphatidylinositol (PI) linkage, we have shown that mAbs to the human CD59 antigen are capable of inducing human T cell proliferation and cytokine production in the presence of phorbol ester and a crosslinking second antibody.

4) In order to probe the cellular mechanisms whereby the administration of the immunosuppressive drug, Cyclosporine A (CsA) induces autoimmune disease, we have established a model in which autoantibodies to gastric mucosal antigens can be induced in the offspring of mothers treated only during pregnancy with CsA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00226-09 LI

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Rabbit Allotypes: Structure, Organization and Regulated Expression of Ig Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Mage	Section Head	LI, NIAID
Others:	B. Newman	IRTA Fellow	LI, NIAID
	N. Hole (to 7/90)	Visiting Fellow/Associate	LI, NIAID
	M. Allegrucci (to 5/90)	Visiting Associate	LI, NIAID
	P. Fuschiotti (from 5/90)	Visiting Associate	LI, NIAID

COOPERATING UNITS *(if any)*
LOM, NIDR (N. Harindranath); Basel Inst. Immunol. Basel, Switzerland (A. S. Kelus); Dept. Microbiol. Loyola Stritch Sch. Med. Chicago (K. Knight)

LAB/BRANCH
Laboratory of Immunology

SECTION
Molecular Immunogenetics Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.88	1.88	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We study genes of the rabbit immune system by techniques of molecular biology coupled with immunological assays. Rabbits of the *Alicia* strain have a mutation *(ali)* that segregates with the immunoglobulin heavy chain (*Igh*) locus and has a cis effect upon the expression of heavy chain variable region genes (VH) encoding the a2 allotype. We have been breeding these animals at the NIH. Genomic DNAs from homozygous mutant and wild type animals were indistinguishable by Southern analyses using a variety of restriction enzyme digests and *Igh* probes. However, in studies of DNA fragments from digests with infrequently cutting enzymes separated by transverse alternating field electrophoresis, we found a relatively small deletion of a segment containing 3' VH genes with important regulatory functions, the loss of which leads to the *ali* phenotype. Our studies of VH gene rearrangement in B-cells suggest that the 3' end of the VH locus probably plays a key role in regulation of VH gene expression in rabbits because VH gene(s) in this region are the targets of preferential VDJ rearrangements. This raises the possibility that alternative mechanisms like gene conversion are at work to generate the antibody repertoire in this species.

We are currently studying five recombinants between immunoglobulin heavy chain variable region (VH) and constant region (CH) genes. Two of the VH-CH recombinants were discovered in our laboratory and three at the Basel Institute for Immunology. In order to localize the sites of the recombinations that led to the new haplotypes, we have been analyzing DNAs from the parental and recombinant haplotypes using a set of probes spanning the VH, DH, JH, $C\mu$ and $C\gamma$ regions of the *Igh* locus. All five recombination sites were downstream of the entire VH gene cluster; three appear to have occurred within the region containing DH genes and one downstream of $C\mu$.

There are two copies of the gene for the rabbit kappa light chain constant region, $\kappa1$ and $\kappa2$. We have now shown that these genes are more than 1 Mb apart and each has associated $V\kappa$ as well as $J\kappa$ genes. We have obtained separated and pure populations of B cells expressing lambda or kappa 2 light chains and shown that the $\kappa1$ gene has been deleted from many of the B cells expressing lambda light chains.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00349-08 LI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Murine Class II MHC Genes and Gene Products

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. Germain, Section Head, LI, NIAID

Others: L. Racioppi, Visiting Associate, LI, NIAID; C. Layet, Visiting Associate, LI, NIAID; R. Koenig, Visiting Associate, LI, NIAID; S. Sadegh-Nasseri, Senior Staff Fellow, LI, NIAID; G. Otten, Special Volunteer, LI, NIAID; L. Hendrix, Research Technician, LI, NIAID; A. Fox, Research Technician, LI, NIAID; L-Y. Yu, Research Technician, LI, NIAID

COOPERATING UNITS (if any)

LCMI, NIAID (R. Schwartz); FDA (L. Matis); Univ. of Chicago (A. Sant); Columbia University (N. Braunstein); Basel Institute for Immunol. (F. Ronchese); Mt. Sinai School of Medicine (E. Bikoff); Metabolism Branch, NCI (J. Berzofsky)

LAB/BRANCH

Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.5	4.0	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Class II MHC (Ia) gene products play critical roles in a variety of T and B lymphocyte responses. A combination of immunological, molecular genetic, and biochemical approaches is being used to study the relationship between Ia structure and function. Sequential intracellular folding and targeted intracellular transport of class II molecules is central to the function of Ia in the acquisition of antigenic peptides for presentation to T cells. These events have been examined using spleen cells and cells transfected with mutant class II α and β chains. Analysis of surface expression has revealed a requirement for "accommodation" of the highly polymorphic regions characteristic of MHC proteins for efficient transport of assembled $\alpha\beta$ dimers to the cell membrane. The interacting regions have been mapped using recombinant α and β chains, and the data provide support for the current structural model of class II molecules based on the class I HLA crystal structure. These studies also have demonstrated that the defect in expression in most cases of "mismatched" α and β chains lies in the failure of assembled heterodimers to efficiently exit the endoplasmic reticulum. Many of these poorly transported dimers can be "rescued" at least in part by co-expression of adequate amounts of the non-MHC encoded invariant chain. Additional studies have revealed that Ia heterodimers adopt a series of conformational states during intracellular transport and that the folding state of Ia molecules is controlled by association with invariant chain and with antigen. The use of direct peptide binding techniques, together with transfectants expressing mutant class II molecules, has permitted the definition of a local peptide binding subregion in the murine I-E molecule, and the role of specific allelically polymorphic residues in controlling the quantitative and qualitative binding of peptide to this molecule. Finally, mutational analysis has begun to define the site(s) of interaction of Ia with CD4, a molecule that functions as a co-receptor along with the T cell receptor in recognition of class II molecules during T cell activation. Together with the studies on class II folding, transport, and control of peptide binding by the polymorphic domain, these experiments will provide new insight into the molecular mechanisms involved in antigen recognition by and activation of T lymphocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00394-07 LI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetic Analysis of Lymphocyte Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	David H. Margulies	Section Head	LI, NIAID
Others:	Lisa Boyd	Chemist	LI, NIAID
	Charles Hoes	Biologist	LI, NIAID
	Maripat Corr	Medical Staff Fellow	LI, NIAID
	Rosemarie Hunziker	Guest Worker	LI, NIAID
	Steven Kozlowski	Medical Staff Fellow	LI, NIAID
	Randall Ribaudo	Guest Worker	LI, NIAID

COOPERATING UNITS (if any)

LI, NIAID (W.-H. Boencke, G. Otten, R. Germain; MB, NCI (T. Takeshita, J. Berzofsky)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	5.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The ongoing focus of the laboratory is to understand in detail the structure/function relationships of cell surface molecules involved in the immune response, employing a combination of methodologies that include molecular biological manipulation of cloned major histocompatibility complex genes, expression analysis of those genes following transfection in various tissue culture cells, expression analysis of those genes following introduction into the germ line of various mouse strains, and functional analysis of purified protein derived from molecularly engineered soluble analogues of these histocompatibility genes. Significant progress over the past year has been achieved in: a) establishing an in vitro system for the pulsing of purified MHC class I protein with antigenic peptide to form a functional MHC/peptide complex; b) the demonstration of the role of β2-m in facilitating the ability of purified MHC molecules to functionally bind antigenic peptide; c) analysis of the biochemical and cellular requirements for the generation of self-tolerance to cell surface molecules using a transgenic mouse system as a model; d) development of systems for studying the interaction of class I molecules by in vitro translation and posttranslational assembly of MHC class I molecules to antigenic peptides; e) development of a baculovirus based expression system for the production of engineered membrane bound and soluble MHC class I molecules; and f) the demonstration that the secretion of engineered soluble MHC class I molecules can be significantly affected by the presence of antigenic peptide.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00403-07 LI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetic Analysis of T Cell Receptor Structure and Repertoire

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. Germain	Section Head	LI, NIAID
Others:	J. Sherman	Medical Staff Fellow	LI, NIAID

COOPERATING UNITS (if any)

FDA (L. Matis); BRMP, NCI (A. Kruisbeek); Chiba University School of Medicine, Japan (T. Saito)

LAB/BRANCH

Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.75	.75	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

T lymphocyte activation occurs through the clonally distributed T cell receptor or via non-clonally distributed cell surface molecules. The available set of αβ receptors is dictated by both positive and negative intrathymic selection events. The molecular basis of this selection, in particular, how bias for self-MHC presented peptides is achieved without corresponding deletions of these clones during establishment of self-tolerance, is unknown. This project uses cellular and molecular tools to study the development of the T cell repertoire and the activation of T cells by αβ and non-clonal surface receptors. The ability of cross-linked, non-clonotypic molecules to activate T cells has been studied using transfected cells. Previous data indicated that for Thy-1 and Ly-6-dependent stimulation, co-expression of TCR-CD3 complexes was required. In contrast, we found with both human cells and transfected mouse cells that anti-CD2 stimulation of IL-2 production can occur in the absence of surface CD3 expression, if the density of CD2 is sufficiently high. The relationship between CD2 level and stimulation is non-linear, and strong synergy is seen with co-expression of TCR-CD3. Mls is an uncharacterized set of molecules able to engage the receptors of T cells bearing certain Vβ-containing TCR. This effect can be seen as intrathymic deletion in mice expressing the Mls product(s), or in the phenotype of proliferating cells from Mls negative mice stimulated with Mls bearing cells. The deletion events involve both CD4+ and CD8+ cells, whereas it was originally thought that stimulation of only CD4 + cells occurred among mature T cells. Recent data suggest CD8+ responses can occur as well. This latter finding raises questions about the ability of anti-CD4 antibody to "rescue" CD8+ cells in the thymus of Mls expressing mice. We have designed experiments to explore whether such cells are functionally tolerant but not deleted in this circumstance. Finally, based on a variety of data, we have proposed a specific molecular model for the intrathymic events of MHC-dependent peptide presentation to and recognition by αβ TCR that underlie the process of positive selection. Tests of this model, together with the above studies, should provide information about how the T cell repertoire develops and the role of various membrane molecules in T cell activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00425-06 LI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Lymphocyte Physiology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T.M. Chused	Senior Investigator	LI, NIAID
Others:	Y.A. Ishida	Visiting Fellow	LI, NIAID
	J.A. Meligeni	Research Associate	LI, NIAID
	E.M. Brown	Biologist	LI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The process of signal transduction across the lymphocyte plasma membrane is under investigation. Novel fluorescent probes of physiologic parameters such as membrane potential, intracellular free ionized calcium, and intracellular pH, in conjunction with the high sensitivity and single cell resolution of flow cytometry, are being utilized. These studies have revealed extensive "feed-forward" and "feed-back" regulatory relationships between ion channel opening, membrane potential, activity of the calcium pump, and rate of phosphatidyl inositol turnover. These mechanisms differ in the T, B and monocyte/granulocyte lineages.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00427-06 LI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigen-Specific and Antigen-Nonspecific Cellular Cytotoxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. V. Sitkovsky	Visiting Scientist	LI, NIAID
Others:	R. Taffs	Staff Fellow	LI, NIAID
	A. Filippini	Visiting Fellow	LI, NIAID
	T. Sugiyama	Visiting Fellow	LI, NIAID
	M. Piper	Research Technician	LI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Biochemistry and Immunopharmacology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To identify proteins (enzymes) involved in the T-cell receptor (TCR)-triggered mechano-biochemical activities of cytotoxic T-lymphocytes (CTL) we studied the biochemical changes in cloned and polyclonal CTL after occupation of functionally important surface proteins by antigens or mAb. The ultimate goal of these studies is to understand molecular mechanisms of cytotoxicity and to design specific peptidic reagents that will modulate the intensity of effector functions of lymphocytes.

Biochemistry and Immunopharmacology Since in our earlier studies we implicated Ca^{2+}/Calmodulin dependent, cyclic nucleotide dependent protein kinases, protein kinase C into "ON" and "OFF" signaling in CTL, we were designing and testing different synthetic peptides substrates, pseudosubstrates and inhibitors of these enzymes to affect effector functions of lymphocytes. We demonstrated the feasibility of such approach in modulation of the functional responses of intact, unpermeabilized cells.

Molecular Mechanisms of Cytotoxicity. We proposed the new model of cell mediated cytotoxicity, the "Extracellular ATP(ATP_o)" hypothesis and demonstrated that ATP_o destroys tumor target cells while CTL themselves are protected by the high levels of ecto-ATPase activity. ATP_o is accumulated by CTL in response to the TCR crosslinking and the addition of ATP_o-degrading enzymes blocks cytotoxicity. In control experiments removal of ATP_o with enzyme does not interfere with the activation of CTL by targets (granule exocytosis response). The presence of ecto-protein kinase activities on both CTL and target cells is demonstrated.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00493-04 LI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

B Cell Stimulatory Factor-1 (BSF-1)/Interleukin-4 (IL-4)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William E. Paul	Chief	LI, NIAID
Others:	S. Z. Ben-Sasson	Visiting Scientist	LI, NIAID
	R. Seder	Research Associate (MSF)	LI, NIAID
	U. Atasoy	NRSA Fellow	LI, NIAID
	A. Keegan	NRC Fellow	LI, NIAID
	M. Plaut	Guest Researcher	LI, NIAID
	T. Tanaka	Visiting Fellow	LI, NIAID

COOPERATING UNITS (if any)

NCI (J. Pierce); USUHS (F. Finkelman, J. Urban); Johns Hopkins University School of Medicine (S. Sharkis)

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.75	1.0	3.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Interleukin 4 (IL-4) is potent lymphokine that regulates a wide variety of functions of lymphoid and other hematopoietic cells. An assay to measure IL-4-production by single cells indicates has been developed. It shows that the frequency of T cells capable of producing IL-4 in lymph node cell suspensions from normal mice is very low, ~1/1000, is found mainly among large T cells and is completely dependent upon the presence of interleukin-2 (IL-2). Lymph node T cells from mice that had been injected with anti-IgD or infected with Nippostrongylus brasiliensis, treatments known to cause an IL-4-dependent increase in serum IgE, show a frequency of IL-4 producing cells five to ten times as great as T cells from naive donors. Although small resting T cells from naive donors have a frequency of IL-4-producing of ~1/2000, they can be "primed" in vitro to develop into IL-4-producing cells by culture with anti-CD3, IL-2 and IL-4; this increases the frequency of IL-4 producing cells to ~1/20. Both IL-2 and IL-4 are required for this in vitro priming method and interferon gamma fails to inhibit such priming.

IL-4 is also made by long term mast cell lines in response to receptor cross-linkage, as are other members of the IL-4 family of lymphokines, notably IL-5, IL-3, and GM-CSF. Among normal spleen and bone marrow cells, a small population of cells have high affinity receptors for IgE. These Fcε receptor positive cells have essentially all the IL-4-producing activity of spleen cells in response to high affinity IgE receptor cross-linkage; frequencies of IL-4 producing cells as high as 1/5 have been achieved. Many of these cells are Alcian Blue positive suggesting that they are either mast cells or basophils. Cells capable of forming mast cell colonies in methylcellulose have a frequency of ~1/10 in this cell population. On a per cell basis, the Fcε receptor positive splenic cells appears to make more than 100-fold more IL-4 per cell than do cells from IL-3-dependent mast cell lines, suggesting that they may be important cells in the mediation of IL-4-dependent events in vivo.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00520-03 LI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of T Lymphocyte Responses to HIV Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Germain	Section Head	LI, NIAID
Others:	W-H. Boehncke	Special Volunteer	LI, NIAID

COOPERATING UNITS *(if any)*

MB, NCI (J. Berzofsky, H. Takahashi, T. Takeshita); LVD, NIAID (B. Moss)

LAB/BRANCH

Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.25	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To develop therapeutic and vaccine strategies to combat HIV-1, the cause of AIDS, it is essential to understand the cell-mediated immune response to this agent. To pursue this goal, murine model systems for immunization, isolation of antigen-specific T cells, and *in vitro* analysis of T cells specific for HIV-1 proteins have been devised. Restimulation of spleen cells from H-2d mice immunized with recombinant vaccinia virus containing the HIV-1 (IIIB) gp160 gene using syngeneic transfected fibroblasts expressing the same gene led to the production of CD8+ cytolytic T cells specific for amino acids 315-329. Such CTL could also be elicited by restimulation of primed spleen cells with peptide alone. Primed CD4+ cells were required, unless the cultures were supplemented with IL-2. This same procedure also was successful in generating CTL specific for the HIV-1 (MN) gp160. Both responses were found in H-2d, but not H-2b or H-2k mice, and the CTL elicited were both H-2Dd restricted. The peptide involved in the MN response corresponded to the same region as that involved in the IIIB response, yet the CTL produced in were highly specific and did not cross-react on target cells incubated with the reciprocal peptide. The difference in specificity could be ascribed to a single residue (position 325, V in IIIB, Y in MN) that varies widely among HIV-1 isolates. These results indicate that this is an immunodominant region of the protein in H-2d mice, possibly because of its dual capacity for stimulation of class I and class II-restricted T cells, and that naturally occurring variation in viral protein structure leads to non-overlapping T cell specificities. This has important consequences for vaccine considerations, and for understanding the selection of virus variants during infection that may escape ongoing immune responses.

Application of the same methodology has led to the identification of a CTL epitope in the *pol* protein, restricted by H-2k. This same determinant (residues 203-219) was found to sensitize targets for lysis by PBL from HIV-infected humans. A similar overlap between murine and human responses was observed for the gp160 epitope, suggesting that studying the murine response to HIV is of potential direct value in the design of human therapeutics.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00545-02 LI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antigen Processing and Presentation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Germain	Section Head	LI, NIAID
Others:	G. Otten	Special Volunteer	LI, NIAID
	J. Sherman	Medical Staff Fellow	LI, NIAID
	D. Weinstein	IRTA	LI, NIAID
	A. Fox	Research Technician	LI, NIAID

COOPERATING UNITS *(if any)*

Molecular Biology Section, LI, NIAID (D. Margulies, R. Ribaudo, S. Kozlowski); Mt. Sinai School of Medicine (E. Bikoff)

LAB/BRANCH

Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.25	1.75	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

T lymphocytes bearing $\alpha\beta$ receptors respond to complexes of peptide antigen and class I or class II MHC molecules. The way in which protein antigens are transformed into peptides suitable for such binding, the events involved in facilitating such peptide-MHC association, and the pathways followed by MHC molecules both before and after peptide association are critical to our understanding of T cell immunity.

We previously proposed two fundamentally distinct pathways for peptide acquisition by class I and class II MHC molecules. Substantial functional data support this model, although important exceptions exist. To examine the biochemical and cell biological basis for this distinction, and the allele-independent molecular events involved in peptide-MHC molecule association, we have examined normal and mutant cells lines varying in their capacity to generate effective peptide-MHC complexes for the rate of class I and class II molecule assembly and transport, in the presence and absence of known MHC ligand peptides, and under varying growth conditions. These studies have revealed that peptide-class I MHC interaction involves what can be considered an "allosteric" interaction among peptide, MHC class I heavy chain, and β2-microglobulin (see also the report of the Molecular Biology Section, LI, NIAID), that results in a stabilization of the ternary complex and resistance to thermal denaturation. This process appears to be localized to the endoplasmic reticulum, and accounts for the predilection of class I molecules for peptides generated from intracellular proteins.

Class II molecules do not share this requirement for peptide for thermally stable chain association, but rather are regulated in their folding and peptide binding capacity by additional molecules, including the invariant chain (see Z01 AI 00349-07 LI). However, in transfected cells, it has not been possible to show a functional role for Ii with respect to the efficiency of antigen processing. These data suggest a more complex role for Ii and specialized physiology in class II-expressing, antigen-presenting cells than previously postulated.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00565-01 LI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Family of κB Regulators for Genes in the Immune Response

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael Lenardo	Senior Staff Fellow	LI, NIAID
Others:	A. Kuang	Visiting Student	LI, NIAID
	K. Novak	Research Technician	LI, NIAID
	S. Kang	Howard Hughes Med. Student	LI, NIAID
	M. Grilli	Visiting Fellow	LI, NIAID
	D. Ghosh	Natl. Library of Medicine Fellow	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Regulation of genes for several lymphokines as well as other molecules involved in the immune response depend on a 10 bp DNA sequence termed, κB. This sequence binds a growing family of nuclear proteins that are capable of tightly governing transcription of these genes. Importantly, the κB sequence is found in the human immunodeficiency virus (HIV). A cardinal feature of the κB sequence is that it permits transcription in a highly regulated fashion-temporally and in appropriate cell-types for specific genes. We are attempting to elucidate how this specific regulation occurs. We have found sequences near the κB sequence in the IL-2 receptor alpha chain genes modulate its activity. They allow it to respond to the transcription factor NK-κB only in T cells. In addition, we have been using a computer program developed by Dr. Ghosh in conjunction with the National Center for Biotechnology Information (National Library of Medicine) to study relationships between gene promoters that contain κB sequences to decipher the co-regulatory signals encoded in the DNA.

We have also found that the microheterogeneity in DNA sequence among κB sites has regulatory significance: We have discovered a novel nuclear factor, termed NF-CYT1 that interacts preferentially with a κB site in the interleukin-2 gene. The presence of NF-CYT1 in a number of different biological conditions is inversely correlated with IL-2 gene expression in T cells. This suggests it may be a negative regulator. Very significantly, this factor binds to the enhancer region of HIV. We postulate it may have a role in suppressing HIV viral transcription in resting T cells.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00566-01 LI

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Gene Regulatory Events in T Cell Tolerance and Thymic T Cell Maturation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael Lenardo	Senior Staff Fellow	LI, NIAID
Others:	S. Kang	Howard Hughes Med. Student	LI, NIAID
	K. H. Lee	Visiting Fellow	LI, NIAID
	A. Chen-Tran	Guest Researcher	LI, NIAID
	H. Dang	Undergraduate Student	LI, NIAID
	P. Salzman	Summer Student	LI, NIAID

COOPERATING UNITS (if any)
LCMI, NIAID (R. Schwartz, B. Beverly, K. Brorson); BRMP, NCI (J. Taubenberger, A. Kruisbeek)

LAB/BRANCH
Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.75	2.5	1.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Increasing evidence suggests important events in T cell maturation in the thymus and tolerance induction are governed by specific gene regulation. Recent studies also suggest T cell tolerance comes about through mechanisms other than clonal deletion. We have been studying a model of clonal T cell inactivation where stimulation of the T cell receptor can lead to a functional inactivation or anergy of the cell. This is manifested by an inability to produce IL-2. Our preliminary studies have shown that anergy is due to abnormalities in nuclear regulatory proteins that lead to faulty IL-2 gene induction.

In the thymus, precursor cells undergo a complex set of developmental events and emerge as T lymphocytes capable of specific antigen recognition. The central molecular controlling events for this process are totally obscure. We are taking several approaches to this problem. First, we have initiated studies of various CD3 chain genes which have different developmental activation profiles. Understanding the nuclear regulatory proteins for these genes may lead to the discovery of primary developmental regulatory proteins. Second, we are generating subtractive cDNA libraries from different stage thymocytes to clone genes for developmental regulatory factors. Third, we will study the role and regulation of IL-2 in the maturation of thymocytes that do not bear antigen receptor. In conjunction with these lines of investigation we will also use thymic regeneration after sublethal irradiation as a clinically relevant model for T cell maturation under conditions in which the thymus has been pathologically perturbed.

PHS 6040 (Rev. 1/84) GPO 914-918

LABORATORY OF IMMUNOPATHOLOGY
1990 Annual Report
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PHS-NIH
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF IMMUNOPATHOLOGY, NIAID
October 1, 1989 to September 30, 1990

Herbert C. Morse III, M.D.
Chief, Laboratory of Immunopathology

Analyses of host-virus interactions continue to be at the forefront of studies in the Laboratory of Immunopathology. As in past years, the major viruses under investigation are murine leukemia viruses (MuLV) and papovaviruses. In studies of MuLV, major emphasis has been placed on developing an understanding of the pathogenesis of retrovirus-induced immunodeficiency using the LP-BM5 MuLV model system. Analyses of papovaviruses have focused on the relationship between class I MHC gene expression and tumorigenicity of adenovirus (Ad) transformed cells, as well as development of new quantal analyses of tumorigenicity of cells transformed by different Ad serotypes, SV40 and polyoma viruses. Other major programs include elucidation of the structure and function of the cbl protooncogene, and dissection of pathways in hematopoietic differentiation with particular emphasis on the B cell lineage.

Two major accomplishments in studies of the murine acquired immunodeficiency syndrome (MAIDS) include molecular cloning and characterization of the etiologic virus, and identification of host genes conferring resistance to disease. The causative agent of MAIDS was found to be a replication defective MuLV (BM5d) that was apparently generated by an initial recombination event between the nonpathogenic replication competent ecotropic MuLV component of the LP-BM5 virus mixture and endogenous MCF-related sequences, followed by a series of deletions in env and pol and mutations in gag to yield a 4.8 kb genome (Chattopadhyay, Morse, Hartley). Using a probe representing 100 bp of BM5d gag region, mRNA from BM5d was shown to be expressed at high levels in lymphoid tissues within 1 week of infection (Pitha, Chattopadhyay, Hartley, Morse) with targets including monocytes and macrophages and both large and small lymphocytes (Fredrickson, Chattopadhyay, Morse, Hartley). New viral integrations in lymphoid tissues were detected by Southern hybridization as early as two weeks post inoculation. In addition, three B cell lineage tumors cultured from infected mice were found to have clonal integrations of and to express BM5d, although oligoclonal populations of B cells found in late stages of MAIDS are not always associated with clonal integrations of the virus (Chattopadhyay, Hartley, Fredrickson, Morse). The cloning of BM5d will permit definition of the portions of the genome responsible for disease induction, and will facilitate the

development of reagents for evaluating the immune response to the virus and for generating vaccinia recombinants.

Inbred strains of mice differ greatly in their susceptibility to induction of MAIDS with some being completely resistant and others dying within 3 months of infection. Predominant determinants of resistance and susceptibility to disease unrelated to nonimmune control of virus spread were found to map within the major histocompatibility complex, with the H-2D locus of $H-2^d$ mice being a major determinant of resistance on some backgrounds. Studies of a broad spectrum of H-2 recombinant and mutant mice revealed that on some backgrounds, other regions of H-2 may interact with D end genes to influence the development of disease and sometimes to control helper virus replication (Makino, Morse, Fredrickson, Hartley). The association of class I genes with resistance may reflect the activity of anti-viral cytotoxic T lymphocytes (Green, Morse), a suggestion strengthened by the finding that mutations of class I genes markedly influence the course of disease (Makino, Hartley, Morse). Finally, studies of the RIIIS strain demonstrated that non-MHC genes can override the influence of H-2 loci. This strain, while bearing "permissive" alleles at loci affecting virus replication, is highly resistant to induction of MAIDS and replication of ecotropic viruses. Crosses with the MAIDS-sensitive, H-2-compatible strain, B10.RIII showed that more than one gene determined the resistant phenotype of RIIIS (Makino, Morse, Hartley). Studies are in progress to more clearly define the relation of class II genes of the MHC to class I-determined patterns of resistance to disease and to elucidate the actions of genes outside the MHC that influence virus spread and pathogenicity.

Studies in the Viral Pathogenesis Section have focused on the mechanisms governing the transforming activities of DNA tumor viruses. Analyses of transformation mediated by DNA viruses have been confounded by the lack of reproducible assays for the function of specific viral genes in relation to the tumorigenicity of cells infected with these agents. A new approach to this problem has been generated in the development of quantal dose-response assays for the oncogenicity of cells transformed by papovaviruses (Lewis). Using this system, several previously unappreciated aspects of tumor growth in hosts with varying levels of immune competence have been defined, including the independence of tumor latency from tumor incidence as a distinct feature of certain tumor lines (Lewis).

Other studies in the Section centered on the relationship of MHC gene expression to the tumorigenicity of adenovirus (Ad) transformed cells. Earlier studies had indicated that cells with reduced class I gene expression as a result of Ad E1A gene expression were more tumorigenic than their counterparts expressing high levels of class I genes. This question was reexamined using Ad-transformed cells expressing high or low levels of class I genes following gene transfection. Unexpectedly, cells expressing high levels of class I genes were more tumorigenic that low expressors,

demonstrating independence of class I gene expression and tumorigenic phenotype (Soddu, Lewis).

Differences in the oncogenicity of different Ad serotypes are well known but poorly understood with Ad2 and Ad5 being nononcogenic and Ad12 highly oncogenic. A basis for understanding the nononcogenic phenotype of Ad5 was provided by the observations that a highly oncogenic hamster cell line was rendered nontumorigenic following transfection with the Ad5 EIA gene in association with markedly enhanced susceptibility of the transfected cell line to NK cell mediated killing (Lewis, Cook).

Other highlights of the year's activities in the Laboratory include:

Characterization of cell-surface markers for B cell and myeloid precursors. A new monoclonal antibody, LIP-6, generated against a tumor with stem cell features was found to recognize a 138 kD protein on the surface of bone marrow cells that have the capacity to differentiate into cells of either the B cell or myeloid lineages in tissue culture. Further characterization of these cells showed them to reside in a minor (1%) population of bone marrow cells that are $CD11b^+$, $CD45R^-$ (Holmes, Morse).

CBL-related sequences in mouse and man. A new nuclear oncogene identified in this laboratory, v-*cbl*, was shown to have two copies of highly related sequences in the mouse genome and one copy in human DNA. The mouse genes were mapped to chromosomes 6 (*Cbl-1*) and 9 (*Cbl-2*) and in man, *CBL2* was mapped to chromosome 11q23. Sequence analyses have shown that *Cbl-1* is a processed gene that inserted in a LINE sequence while *Cbl-2* is a complex gene with primary transcripts of 11 and 3.7 kb (Shapiro, Langdon, Regnier, Morse).

Administrative changes. Ms. Nan Lofgren joined the Laboratory as Secretary to the Chief. Dr. Marjorie Shapiro moved into a Staff Fellow position. Dr. Eggerton Campbell joined the Laboratory in an IRTA position and Dr. Ambros Hugin joined the Laboratory as a Visiting Associate. Dr. Daniel Regnier left his Fogarty position for an appointment in Nice, France.

Honors and awards. Dr. Herbert C. Morse III was named as an associate editor of the journal *In Vivo* and was elected to membership in the American Association of Pathologists.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00013-27 LIP

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunobiology and Pathogenesis of DNA Virus Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A.M. Lewis, Jr.	Head Viral Pathogenesis,	LIP, NIAID
Others:	S.Soddu	Visiting Fellow,	LIP, NIAID
	A.S. Levine	Scientific Director,	OSD, NICHD

COOPERATING UNITS (if any)

National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado (J.L. Cook).

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Pathogenesis Section

INSTITUTE AND LOCATION

NIAID. NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	1.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The difficulties of trying to evaluate the complexities of those cell-host interactions that result in either success or failure of sustained tumor growth interferes with a better understanding of viral carcinogenesis. The tumorigenic phenotypes expressed by DNA virus transformed cells represent the end product of those oncogene controlled events that are involved in cell transformation. Differences in these phenotypes provide a window for observing viral oncogene functions. By studying tumor cell dose-tumor incidence relationships and including the time of tumor appearance, we have developed a standard, quantal format for modeling viral transformation and analyzing oncogene-determined tumorigenic phenotypes. The high oncogenicity of certain tumor cells, including adenovirus 12 induced tumor cells, has been related to their expression of low levels of class I MHC proteins. We have used our quantal format to study Ad12 transformed BALB/c mouse cells that express low or high (subsequent to syngeneic class I gene transfection) class I MHC protein levels. Low expressor cells are highly immunogenic in CTL mediated assays of virus-specific immunogenicity and are rejected as allografts by H-2 incompatible mice. High class I expressors are also immunogenic and are quantally more tumorigenic than their low expressor parents. Thus, high oncogenicity of BALB/c mouse cells does not depend on expression of class I MHC proteins. An Ad2/Ad5 E1A ongene-controlled pathway that leads to heightened susceptibility to killing by NK cells exists in Ad2/Ad5 infected and transformed cells. Such a pathway may exist in highly aggressive tumor cells. Evidence for such a pathway was obtained by showing that expression of Ad5 oncogene in sarcoma cells eliminates their tumor inducing capacity by activating their susceptibility to NK cell killing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00135-16 LIP

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Properties of Immunoglobulin Secreting Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H.C. Morse III	Chief	LIP, NIAID
	M.A. Principato	Investigator	LIP, NIAID
	U.R. Rapp	Senior Investigator	LVC, NCI
	J.H. Pierce	Senior Investigator	LCMB,NCI
	K.L. Holmes	Senior Investigator	BRB, NIAID

COOPERATING UNITS *(if any)*
University of Western Australia (S.P. Klinken)

LAB/BRANCH
Laboratory of Immunopathology

SECTION
Virology and Cellular Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Analyses of the pathways taken by multipotential progenitor cells in giving rise to diverse elements of the hematopoietic system are poorly understood. The relationship between different pathways of hematopoietic differentiation has been investigated using in vivo transformation of bone marrow cells with retroviruses containing v-myc alone, v-raf alone or the two oncogenes in a single retrovirus construct. Viruses containing the single oncogenes were found to induce only B cell lineage tumors while the dual-oncogene virus induced cell lines with the ability to differentiate along two different pathways, yielding mature macrophages or mature B cells. These results support earlier suggestions of a close relationship between the myeloid and B cell differentiative pathways.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00138-15 LIP

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Viruses and Immune Response

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. C. Morse, III	Chief,	LIP, NIAID
Others:	J. W. Hartley	Senior Investigator	LIP, NIAID
	K. L. Holmes	Investigator	LIP, NIAID
	T. N. Fredrickson	Research Microbiologist	LIP, NIAID
	R. M. L. Buller	Senior Staff Fellow	LVD, NIAID
	A. Singer	Chief	LEI, NCI

COOPERATING UNITS (*if any*)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Inactive this year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00205-09 LIP

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Abnormalities of T and B Lymphocytes of Autoimmune Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. C. Morse, III	Chief	LIP, NIAID
Others:	W. F. Davidson	Visiting Scientist	LG, NCI
	C. Calkins	Guest Researcher	LIP, NIAID

COOPERATING UNITS *(if any)*

Juntendo University, Tokyo, Japan (T. Shirai, K. Okumura)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Inactive this year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00284-09 LIP

PERIOD COVERED

October 1, 1990 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Pathogenic Murine Leukemia Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. W. Hartley	Hd, Viral Oncology Section,	LIP, NIAID
Others: H.C. Morse III	Chief	LIP, NIAID
T.N. Fredrickson	Research Microbiologist	LIP, NIAID
K.L. Holmes	Senior Investigator	LIP, NIAID
S.K. Chattopadhyay	Expert	LIP, NIAID
M. Makino	Visiting Fellow	LIP, NIAID
S. Ruscetti	Investigator	NCI

COOPERATING UNITS (if any)

Massachusetts Institute of Technology, Cambridge, MA (N. Hopkins, N. Speck); American Red Cross, Bethesda, MD (G. Jay); Univ. of California, Davis (S. Hinrichs).

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.1	1.3	2.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project concerns the isolation and biological and molecular characterization of C-type murine leukemia viruses (MuLVs). Present emphasis has been on analysis of the mechanism of action of the defective MuLV genome (BM5def) identified as the pathogenic element in LP-BM5 virus mixtures, which also contain replication competent, B-tropic ecotropic and MCF MuLVs. Infection of susceptible mice by LP-BM5 MuLV results in lymphoproliferation and progressive impairment of normal functions of the immune system involving both T- and B-cells, a syndrome termed murine AIDS (MAIDS). Pathogenesis involves interactions between T- and B- cells and with non-T, non-B lymphoid cells in which the role of expression of BM5def and helper virus has not been precisely defined. Restriction endonuclease mapping, molecular cloning and partial sequencing of BM5def and BM5eco, and immunoprecipitation analyses show that the 4.9Kb defective genome contains major deletions in the <u>env</u> and <u>pol</u> regions, as well as alterations in the p12 region of <u>gag</u> resulting in an unusual 60-kDa precursor protein. By Southern blot analysis mouse cellular DNAs were found to exhibit strain-specific patterns of bands hybridizing with a probe for BM5def sequences. With appropriate restriction enzymes an extra-genomic 4.4Kb band has been detected in B-cell tumor lines and lymphoid tissues derived from infected mice, and studies are in progress to define the time of appearance and tissue specificity of viral integration and mRNA expression. In other, collaborative, studies it was found that subtle structural alterations in the highly conserved MuLV enhancers can alter disease phenotype; and the development of olfactory neuroblastoma in mice transgenic for adenovirus E1 genes was found to be associated with activation of endogenous MuLV.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00286-09 LIP

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of Genetic Control of Murine Leukemia Viruses and Virus-Induced Neoplasms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. W. Hartley	Hd, Viral Oncology Section	LIP, NIAID
Others:	M. Makino	Visiting Fellow	LIP, NIAID
	H.C. Morse III	Laboratory Chief	LIP, NIAID
	T.N. Fredrickson	Research Microbiologist	LIP, NIAID

COOPERATING UNITS (if any)

R. Schwartz, NIAID; D. Sachs, NCI, D. Murphy, NY State Dept. of Health; I. Egorov, Jackson Labs; C. David, Mayo Clinic; M. Zijlstra, Whitehead Inst.

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.1	1.6	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

LP-BM5 MuLV induces in C57BL and other sensitive mouse strains a syndrome of lymphoproliferation and severe immunodeficiency, termed "MAIDS". The causative virus is a replication-defective MuLV which usually requires "help" by competent B-tropic MuLVs, for which Fv-1b mouse strains, like C57BL, are permissive. Strain distribution patterns in inbred mice for sensitivity and resistance to MAIDS indicate major host influences associated with alleles at Fv-1, genes of the H-2 complex, and non-MHC related genes. Influences on disease reflect regulation of helper virus infection and spread, class I MHC control, and class II MHC associated steps in pathogenesis. The most sensitive mice among <u>Fv-1b</u> strains are of the <u>H-2b</u> haplotype (H-2 haplotypes f,j,k,p,q,r and s are also associated with sensitivity) while <u>Fv-1b</u> <u>H-2d</u> strains are highly resistant. Mapping studies using intra-H-2 recombinants and transgenic mice indicate that H-2d-mediated resistance is clearly linked to the H-2D end of the region if the rest of the MHC is derived from the b haplotype. Expansion of these studies to explore the mechanism of D end resistance, to evaluate other regions of the MHC, and to clarify the pathogenesis of MAIDS reveal a very complex interaction of H-2 region genes, both class I and class II in determining degree of sensitivity or resistance. For example, F-1 hybrids of sensitive and resistant strains are sensitive, suggesting a Class II-like susceptibility; mice of s or q haplotype which are d at H-2D are not resistant although perhaps not fully sensitive; and mutations in major histocompatibility genes at H-2K can result in acceleration or delay in the disease process. Despite the B-tropism of the ecotropic helper virus in LP-BM5 certain Fv-1n strains have been found to be relatively sensitive to MAIDS, the mechanism apparently involving utilization of endogenous MuLV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00465-05 LIP

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Retrovirus-induced Murine Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. C. Morse III	Chief	LIP, NIAID
Others:	J. W. Hartley	Senior Investigator	LIP, NIAID
	M. Makino	Visiting Fellow	LIP, NIAID
	G. Shearer	Investigator	LEI, NCI
	J. R. Wunderlich	Investigator	LEI, NCI
	K. L. Holmes	Investigator	BRB, NIAID
	S. K. Chattopadhyay	Expert	LIP, NIAID

COOPERATING UNITS *(if any)*

National Institute of Health, Tokyo, Japan (T. Mizuochi)
Dartmouth Medical School (W.Green); Veterans Administration Medical Center, Baltimore, MD (R.A. Yetter)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mice infected with a mixture of retroviruses, termed LP-BM5 murine leukemia viruses (MuLV), develop an immunodeficiency and lymphoproliferative syndrome with many similarities to human AIDS. We have investigated cellular interactions that contribute to the development of these abnormalities. One of the central features of this disease is dysfunction of CD4+ T cells. It was shown that *in vivo*, development of phenotypic and functional abnormalities of this T cell subset is dependent on the presence of mature B cells. *In vitro* analyses of this phenomenon suggested that several mechanisms may contribute to induction of this abnormality. First, normal CD4+ T cells providing help for self + X cytotoxic T lymphocyte (CTL) responses were found to be actively inhibited by cells from infected mice, suggesting induction of suppressor cells following infection. Second, antigen specific T cell clones were shown to proliferate poorly in response to antigen presented by spleen cells from infected mice suggesting impaired function of antigen presenting cells (APC). Finally, we demonstrated that CD4+ T cells primed *in vivo* to soluble antigens were impaired in their ability to generate secondary *in vitro* proliferative responses, suggesting an acquired intrinsic defect of CD4+ cells. In other studies, we have found that an active anti-viral CTL response can be generated in mice susceptible to the disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00484-04 LIP

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms in Hematopoietic Cell Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K.L. Holmes	Hd, Flow Cytometry Section	BRB, NIAID
Others:	H.C. Morse, III	Chief	LIP, NIAID
	D. Klinman	Investigator	CBER,FDA
	M. Makino	Visiting Fellow	LIP, NIAID

COOPERATING UNITS (if any)

George Washington University School of Medicine, Washington DC (J. Albright)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A major emphasis has been on the identification and isolation of early B and myeloid cell progenitor populations in murine bone marrow (BM) utilizing cell surface antigen expression and flow cytometric analysis and sorting. The further characterization of the antigen recognized by the monoclonal antibody, LIP-6 has shown that in addition to its expression on mature B lymphocytes and macrophages, it is also found on cells capable of differentiating in vitro into B cells and myeloid cells. This population, representing approximately 1% of BM, also expresses Mac-1(Ly-40;CD11b), which we have previously shown to be expressed on early B and myeloid progenitors.

Studies of the unique B cell subset, Ly-1(CD5)$^+$ B cells, has shown that its expressed antigen repertoire differs according to whether the cells are isolated from the peritoneum vs. the spleen. Phenotypic studies of peritoneal Ly-1(CD5)$^+$ B cells has demonstrated heterogeneity in the expression of a pan-B cell antigen, Lyb-2 (recently found homologous to CD72). This Lyb-2$^+$ population appears to secrete more antibody in response to LPS and IL4, as compared with the Lyb-2$^-$ B cells.

New studies of the pan-B antigen, Lyb-8.2, show that it is expressed late in B cell differentiation and preliminary results suggest that it is closely associated with IgM and IgD on the cell surface.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00544-02 LIP

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Basis of Pathogenesis of Murine Leukemia Virus Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S.K. Chattopadhyay	Expert	LIP, NIAID
Others:	H.C. Morse III	Chief	LIP, NIAID
	J.W. Hartley	Hd, Viral Oncology Sec.	LIP, NIAID
	T.N. Fredrickson	Research Microbiologist	LIP, NIAID
	M. Makino	Visiting Fellow	LIP, NIAID
	S.K. Ruscetti	Investigator	NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	0.3	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A mixture of murine leukemia viruses (MuLV), termed LP-BM5 MuLV, induces a syndrome characterized by lymphoproliferation and immunodeficiency (MAIDS) in susceptible strains of mice. The components of this virus mixture have been characterized by combined biologic and molecular cloning techniques. Biologically cloned ecotropic MuLV and a series of MCF MuLV did not induce MAIDS alone or in combination, suggesting the activity of another virus component. Molecular genetic analyses of the virus mixture revealed the presence of an additional 4.9 kb defective genome that was regularly associated with the development of disease. The ecotropic and 4.9 kb molecular species were molecularly cloned and shown, in combination, to induce changes characteristic of MAIDS. Sequence analyses of the 4.9 kb genome demonstrated that it was most likely derived by an initial recombination between the ecotropic virus and endogenous MCF-like sequences followed by deletion and mutation. The most highly conserved region of the 4.9 kb virus in relation to the ecotropic parent is in <u>gag</u> with the aminoterminal portion of p12 being the most divergent. Further studies will be directed at determining the molecular features of the defective genome responsible for MAIDS induction.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00578-01 LIP

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of Oncogenes in B Cell Neoplasia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M.A. Shapiro	Senior Staff Fellow	LIP, NIAID
Others:	H. C. Morse, III	Chief	LIP, NIAID
	J. F. Mushinski	Investigator,	LG, NCI
	C. A. Kozak	Research Microbiologist	LIP, NIAID
	D. Regnier	Visiting Fellow	LIP, NIAID

COOPERATING UNITS (if any)

Adelaide (W.Y. Langdon); Bowman Gray School of Medicine (P. Savage); University of Minnesota (J. Kersey); Temple University (C. Croce)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	0.3	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The development of neoplasms in vertebrates is often associated with alterations in the structure or expression of cellular proto-oncogenes. We have isolated, cloned and sequenced an oncogene, v-cbl, that was captured by a retrovirus that induces pre-B cell lymphomas in vivo. Cellular sequences related to v-Cbl map to mouse chromosomes 6 (Cbl-1) and 9 (Cbl-2) and to human chromosome 11. Studies of human neoplasms carrying various translocations of chromosome 11 have mapped the cbl gene to 11q23 in close linkage to the THY1 and ETS1 loci. We have isolated genomic and cDNA clones of Cbl and are sequencing these clones to determine the structure of the CBL gene in normal cells. The Cbl-1 gene is a pseudogene containing many mutations with respect to the cbl cDNA sequence and it was inserted into a LINE element. Sequencing of the Cbl-2 locus shows this gene to have a complex organization since the exons are small (88-250 nucleotides) and size of the mRNA is quite large with 2 predominant mRNA species of 11 and 3.7 kb. Cbl mRNA was found to be transcribed in resting fibroblasts and was not inducible in response to various stimuli. Studies with antibodies against v-cbl show cbl to be a nuclear protein. In addition, cbl is homologous to GCN4, a yeast transcription activation factor, in its DNA binding and transactivation regions. These data suggest that cbl may also be a DNA binding transcription factor. Further studies will be directed at completing the sequence of the Cbl-2 germline gene to elucidate its structure, and to determine the 5' regulatory region of the gene to evaluate which transcription factors play a role in cbl expression.

LABORATORY OF IMMUNOREGULATION
1990 Annual Report
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Summary Report
Laboratory of Immunoregulation
October 1, 1989 through September 30, 1990

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Chief, Laboratory of Immunoregulation

Immunopathogenic Mechanisms of HIV Infection

Over the past several years, the LIR has been involved in studying the mechanisms of immunopathogenesis of human immunodeficiency virus (HIV) infection. We have concentrated particularly on the cellular and molecular mechanisms involved in the regulation of HIV expression in chronically infected T lymphocytic and promonocytic cell lines as well as primary cultures of monocyte derived macrophages (MDM) and T cells. We have demonstrated that HIV expression can be modulated by a group of cytokines which play an important role in the normal homeostasis of the immune system. In particular, we have demonstrated that tumor necrosis factor alpha and beta (TNF-α and β) can upregulate HIV expression in T cell lines (ACH-2) via activation of the cellular transcription factor NF-κB which binds to an NF-κB binding sequence of the promotor region of the HIV LTR, and similar mechanisms are also operable in chronically infected promonocytic cells (U1). Furthermore, we observed that TNF-α can upregulate HIV expression in primary MDM infected *in vitro*, suggesting that this cytokine can act on a variety of infected cells of different lineages and at different stages of maturation/activation. We also demonstrated that other cytokines such as interleukin (IL)-6, granulocyte/macrophage-colony stimulating factor (GM-CSF) alone and in synergistic combination with each other and TNF-α can induce HIV expression in U1 cells as well as in primary MDM. Of note is the fact that IL-6 and GM-CSF act predominantly at a post-transcriptional level, while their synergistic interaction with TNF-α results in increased HIV transcription. Since these cytokines play major roles in the regulation of the normal immune response, their ability to regulate HIV expression is of potential importance in progression of HIV-induced immunosuppression.

We have also demonstrated that glucocorticoids (GC) are post-transcriptional co-inducers of HIV expression in U1 cells with IL-6, GM-CSF, and interferon-gamma (INF-γ). In addition, heat shock (42°C) directly induces HIV expression in U1 and ACH-2 cells by transcriptional activation involving NF-κB binding. Furthermore, physiologic heat (40°C) synergizes with IL-6, GM-CSF, and GC in upregulating virus expression in U1 cells.

In contrast to the ability of certain cytokines to induce HIV expression, other cytokines can downregulate virus. In addition to INF-α, the pleiotropic cytokine transforming growth factor-beta (TGF-β) suppresses the induction of HIV by phorbol myristate acetate (PMA) but not by TNF-α. Since endogenous TNF-α production plays a role in the PMA-induction of HIV, the ability of TGF-β to block PMA induction of virus but not induction by exogenous TNF-α allowed us to dissect out the TNF-dependent and TNF-independent components of the PMA induction of HIV expression. Of note is the fact that in contrast to the lack of effect on TNF-α, TGF-β does suppress the induction of HIV expression in U1 cells by IL-6 and GM-CSF either alone or in synergistic combination. It also suppresses HIV replication in primary *in vitro* infected MDM. TGF-β suppresses HIV expression at both the transcriptional and post-transcriptional levels.

The physiologic relevance of cytokine regulation of HIV expression was demonstrated in studies of the role of activated B cells from HIV infected individuals in the induction of HIV expression in infected T cells and monocytes. Activated B cells secrete increased amounts of TNF-α and IL-6 which are capable of inducing HIV expression in U1 and ACH-2 cells. B cells from HIV infected individuals are activated *in vivo* and constitutively secrete IL-6 and TNF-α. These cytokine enriched supernatants as well as the B cells themselves in co-culture are potent inducers of HIV expression. Since activated B cells exist in close proximity to infected T cells in the lymph nodes of HIV-infected individuals, this finding has potentially important implications in the *in vivo* induction of HIV expression.

We have previously demonstrated by polymerase chain reaction (PCR) gene amplification that the reservoir of HIV in the peripheral blood is the $CD4^+$ T lymphocyte. HIV provirus is present in a frequency of 1/10 to 1/100 $CD4^+$ T cells in AIDS patients and from 1/100 to 1/100,000 in asymptomatic seropositive individuals. We have recently demonstrated a direct correlation between increasing virus burden, progressive decline in $CD4^+$ T cell counts and clinical deterioration. In fact, in asymptomatic HIV-infected individuals with comparable numbers of $CD4^+$ T cells, the viral burden at entry of study is predictive of a deterioration of immune status and clinical course. We further demonstrated that the memory subset ($CD29^+$, $CD45RO^+$) of $CD4^+$ T cells was preferentially, but not exclusively infected with HIV as compared with the naive subset ($CD45RA^+$) both *in vitro* and *in vivo*. These observations may explain in part the selective defect in antigen responsive T cells in HIV infected individuals since the antigen responsive T cell is contained in the memory subset. Finally, we demonstrated that intrathymic T cell precursors as early in ontogeny as the "triple negative" stage, i.e. $CD3^-/CD4^-/CD8^-$ cells can be infected with HIV. In fact, even these so-called triple negative cells express sufficient amounts of CD4 molecules on their surface to allow for infection of HIV. These observations have important implications in understanding the lack of ability of the $CD4^+$ T cell pool to regenerate itself even in the face of suppression of virus replication by anti-viral agents, as well as in strategies for immunologic reconstitution.

We have demonstrated that the HIV-specific cytotoxic T cell is contained predominantly in the $CD8^+/DR^+$ subset and that this subset of cells is deficient in its clonogenic potential. Over the course of HIV infection, individuals experience a progressive and preferential loss of HIV-specific cytolytic function whereas broad cytolytic potential remains intact. This progressive decrease in the pool of HIV-specific cytolytic T cells may be due to the fact that this $CD8^+/DR^+$ subset has a decreased ability to expand. Furthermore, we have demonstrated that $CD8^+$ T cells can be infected *in vitro* and *in vivo*. In the *in vitro* experiments, contact of $CD8^+$ T cells with infected $CD4^+$ cells was required for infection of the $CD8^+$ cells since free virus was incapable of infecting the cells. The $CD8^+$ T cells could serve as a reservoir for HIV and could pass on the infection to other $CD4^+$ cells. The close contact in lymphoid tissues between infected $CD4^+$ cells and HIV-specific $CD8^+$ cytolytic T cells may account for the infection and resulting functional impairment and/or depletion of these cells *in vivo*.

In studies designed to assess the mechanisms of action of agents designed to block spread of HIV, we first demonstrated that recombinant (r) CD4 inhibited virus replication even when spread of HIV occurs predominantly by cell to cell contact. We have also demonstrated that monoclonal antibodies directed against cell adhesion molecules LFA-1 and LFA-3 inhibit virus replication by interfering with cell to cell transmission of HIV. More importantly, we demonstrated that the combination of suboptimal concentrations of rCD4 and anti-LFA-1 antibody resulted in a synergistic and enduring suppression of HIV replication.

Furthermore, by using rCD4 and LFA-1 we have observed an interesting dissociation among syncytia formation, cytopathicity, and virus replication. (Schnittman, Poli, Bressler, Stanley, Pantaleo, DeMaria, Butini, Lane, Koenig, Fox, Kehrl, Rieckmann, Kalebic, Thevenin, Graziosa, Biswas, Fauci, LIR/NIAID).

In order to dissect the molecular mechanisms by which HIV kills $CD4^+$ T cells, we have engineered T cell lines expressing HIV envelope glycoproteins and examined their pattern of tyrosine phosphoprotein substrates. We found that the expression of functional gp120 and gp41 had no effect on cell growth and was not cytotoxic to $CD4^+$ T cells. We further showed that envelope protein expression did not alter the pattern of tyrosine-phosphorylated proteins in these cell lines. In contrast, HIV infection of $CD4^+$ cells led to increases of two tyrosine-phosphorylated proteins of 95 and 40 kilodaltons, but phosphoproteins pp56 and pp59 probably corresponding to the T cell activation proteins lck and fyn, respectively, were unaltered. In additional studies, the antibody genes from two human anti-gp41 B cells were cloned as the initial step in developing a chimeric anti-HIV T cell receptor. Certain of the envelope-transfected cell lines, lacking expression of tat and of the recently-described tev genes, predominantly synthesized a truncated p75 envelope-related protein as an alternative to normal gp120/gp41 production. This truncated protein is not associated with infectious HIV virions, and understanding its production may provide an opportunity to attack HIV infection with a new strategy. (Cohen, Tani, Lane, LIR/NIAID).

Regulation of HIV Gene Expression

Using site-directed mutagenesis and computer analysis we identified 14 regions in CAR/RRE (the RNA target sequence of the HIV-1 rev protein) which were base-paired to form seven stem/loop structures. Mutagenesis also indicated that several regions of CAR/RRE were unnecessary for function and more recently has indicated that several regions may be involved in sequence-specific recognition by factors critical for rev regulation. Band shift analysis of the binding of rev protein to CAR/RRE RNA has tentatively identified several regions essential for rev binding and several regions which are probably required for the binding of cellular factors. Further studies on CAR/RRE have indicated that it may be modified by the dsRNA unwindase/adenosine deaminase activity present in all eukaryotic nuclei. In unrelated studies we have developed evidence that gag may have important effects on the cellular tropism of HIV-1. (Dayton, Dayton, Powell, LIR/NIAID; Klotman, Daefler, LTCB/NCI; Nishikura, Wistar Inst.; Baroudy, Gamble Inst).

Neuropathogenesis of HIV Infection

Human astrocytes were grown in culture and shown to secrete molecules which stimulated HIV-1 expression in a chronically infected promonocytic clone, U1.1.5. Interleukin-6, shown by others in the laboratory to synergize with TNF-α in stimulating HIV-1 expression in U1.1.5 cells, was detected in human and rat astrocyte-conditioned media. TGF-β was detected in herpes zoster virus-infected human brain and in human and rat astrocytes exposed to HIV-1 *in vitro*. IL-1 was found to stimulate TGF-β expression in cultured rat astrocytes, microglia and oligodendrocytes. This stimulation was regulated post-transcriptionally in a time- and dose-dependent manner in primary rat astrocytes. These cells were also shown *in vitro* to secrete and bind endothelin-3 a potent vasoconstrictor. Exposure of rat granule neurons to HIV-1 resulted in their death. Brain isolate of HIV-1 BRVA, was the most potent isolate tested in the destruction of neurons and neurites. Taken together, these results suggest that both direct and indirect mechanisms may operate in the

neuropathogenesis of HIV-1 infection and that the indirect mechanism may involve astrocytic cytokines. (Vitkovic, daCunha, Ehrenreich, Fauci, LIR/NIAID).

The Immune Response to HIV and Related Retroviruses

Our efforts have been concerned primarily with cell mediated immune responses to the two human retroviruses causing diseases in man, namely human T lymphotropic virus-1 (HTLV-1) and HIV. We have demonstrated that chronic infection with either virus may result in high levels of circulating cytotoxic T cells (CTL) that can be detected in freshly separated peripheral blood mononuclear cells (PBMC). These CTL are $CD8^+$ and major histocompatibility Class (MHC) I restricted. In individuals infected with HIV, CTL activity is found more often in healthy patients with stable $CD4^+$ counts, particularly in the subset of these patients with detectable levels of p24 antigen in their sera. CTL activity appears to decline with clinical progression to AIDS. In contrast, in patients infected with HTLV-1, high levels of these circulating CTL are only found in patients with neurological disorders, particularly individuals with tropical spastic paraparesis (TSP). CTL in HIV-1 infected individuals are primarily generated against structural proteins, while in patients with TSP, the predominant response is against products of the pX region. Using limiting dilution cloning techniques and mitogenic stimulation, we have generated HIV-1 and HTLV-1 specific CTL clones. Using synthetic peptides, we have mapped two new CTL epitopes within HIV-1 gag and nef proteins. We have demonstrated that CTL epitopes to HIV-1 proteins can be mapped directly using freshly separated PBMC. We have also shown that a synthetic peptide containing the correct epitope may not be recognized by CTL due to surrounding amino acid sequences that may influence binding to HLA Class I and recognition by the T cell receptor. We have noted that CTL to nef can lyse HIV infected cells and such clones can inhibit HIV replication *in vitro*. We have found that if these clones are activated with peptides containing the appropriate epitope, inhibition of viral replication can occur through the release of soluble products from these cells, obviating the need for contact with $CD4^+$ cells harboring HIV. We have also defined a method for mapping B cell epitopes that are recognized by antibodies mediating antibody-dependent cellular cytotoxic responses (ADCC) and have shown that high levels of circulating $CD16^+$ cells are armed with these antibodies *in vivo*. (Koenig, Wood, Fauci, LIR/NIAID; Moss, Earl, Karacostas, LVD/NIAID; Jacobson, McFarlin, NI/NINDS; Shida, Kyoto Univ; Fuerst, Woods, Newell, Brewah, Molecular Vaccines, Inc.).

Clinical Trials in HIV Infection

A series of clinical trials have been carried out in patients with HIV-1 infection. A placebo controlled trial of IFN-α demonstrated benefit to patients with early stage HIV infection and these observations are being followed up in a trial comparing treatment with zidovudine to treatment with interferon-alpha to combination therapy with both drugs in patients with early stage HIV-1 infection. Phase I trials of 3'-azido-2',3'-dideoxyuridine and recombinant CD4 immunoglobulin failed to demonstrate surrogate marker evidence of efficacy. In the area of treatment of opportunistic infections, a masked, randomized controlled trial of foscarnet in patients with HIV-I infection and cytomegalovirus (CMV) retinitis demonstrated activity of this agent not only against CMV, but against HIV as well.

A phase I trial of gp160 as a candidate HIV-1 vaccine was carried out in 125 seronegative high risk individuals. Antibody responses to the vaccine were dose dependent with the highest specific antibody levels occurring after the 1 year booster immunization. The antibodies generated in response to this immunogen react with several of the important

neutralizing domains of the HIV-1 envelope and are capable of inhibiting syncytia formation at titers of 1:800. Group specific T cell proliferative responses to gp160 were noted at all doses, with a peak response occurring at 1 dose level lower than the dose giving the peak specific antibody response.

A variety of approaches were taken in an attempt to improve the immunologic function of patients with HIV-I infection. Active immunization with gp160 was capable of increasing the number of circulating T lymphocytes responsive *in vitro* to gp160. Bone marrow transplantation in combination with zidovudine resulted in only transient improvements in immune function. Trials using GM-CSF or IL-2 in combination with zidovudine and/or IFN-α demonstrated the potential value of combination regimens employing biologic response modifiers in the treatment of patients with HIV-1 infection. (Lane, Fauci, R Davey, Zunich, Zurlo, Easter, Lee, V Davey, Metcalf, LIR/NIAID; Masur, Kovacs, Falloon, Polis, Henderson, CC/NIH; Nussenblatt, NEI).

International AIDS Activities

In a survey of 12,032 employees and their spouses at two large businesses in Kinshasa, Zaire, 4.5% were seropositive for HIV-1 and had a 0.5% annual seroincidence predominately due to heterosexual transmission. In this study, AIDS continued to be the leading cause of death among employed individuals, responsible for 50% of deaths. We also demonstrated that 40% of 1200 female prostitutes in Kinshasa were HIV-1 seropositive and that incident infections (3%/yr) were strongly correlated with genital ulcers and chlamydial infections. Similar associations with HIV-1 infection and genital ulcerative diseases were confirmed in our studies among women in Haiti, and patients attending a STD clinic in Baltimore. In studies of perinatal infection in Zaire, Kenya and Haiti, we documented a 25-35% transmission rate in HIV-1 infection, and resulted in a cumulative mortality rate of 70% in the first two years of life. Of surviving children during the first year of life a beneficial effect of immunizations was observed despite the lower immunogenicity in HIV-1 seropositive individuals. In virologic studies we demonstrated the presence of HIV-1 genome by PCR and/or culture in 5% of 101 seronegative high risk individuals confirming a prolonged latency period before seroconversion. HIV-1 antigenemia was also observed to be decreased in Africans and U.S. blacks, probably secondary to p24 antibody-antigen complexes secondary to increased p24 antibody levels. Additional studies also demonstrated the presence of neutralizing antibody to the V3 loop region of HIV-1_{nm} in HIV-1 infected individuals in the U.S., Zaire and Brazil suggesting the importance of this particular epitope of HIV-1_{nm}. Serologic screening for HTLV-I/II demonstrated increasing rates of HTLV-I infection among Africans and blood transfusion recipients in the U.S., and HTLV-II among IV drug users. (Quinn, Brown, Fauci, LIR/NIAID; Ryder, CDC; Piot, Colebunders, Institute of Tropical Medicine, Antwerp, Belgium; Hook, John Hopkins).

Isolation and Characterization of Genes Important in Human B Cell Function.

Using subtractive hybridization techniques we have identified a group of genes which are expressed in B cells and not in T cells. One of these genes originally identified as clone 66 has been used to isolate several full length cDNA clones, 2 of which have been fully sequenced. The predicted protein from the clone 66 cDNA has a predicted protein core of 95 kilodaltons and has 12 n-linked glycosylation sites with a predicted molecular weight of 140 kilodaltons. Analysis of the predicted protein suggested that it is an integral membrane protein with an intracytoplasmic portion of 140 amino acids. Search of NBRF protein data base revealed a significant homology with 2 homotypic cell adhesion proteins,

carcinoembryonic antigen and myelin associated glycoprotein. The clone 66 cDNA was subcloned into two different mammalian expression vectors. One of the constructs was used to transiently express clone 66 in COS cells. Functional studies revealed that clone 66 encoded for the membrane antigen defined by CD22 and that tonsillar B cells adhered to the COS cells expressing clone 66. The other construct was used to permanently transfect a CD22 negative B cell line. This cell line is now CD22 positive and has altered its growth pattern growing as an adhesive cell line, confirming our results with the transient transfection. We have renamed this B lymphocyte cell adhesion molecule as BL-CAM.

We have continued our studies with several other clones isolated by subtractive hybridization and sequencing and expression studies are in progress. In addition we have isolated cDNAs for 3 homeotic genes from a B cell cDNA library. One of these clones was identical to a previously isolated human homeotic gene, HOX 2.3; however, the other 2 cDNAs were found to encode for previously unrecognized homeobox containing proteins which were designated HB9 and HB24. All three of these genes were found to be present in activated B cells but at very low levels in unactivated B cells. The activation of HB9 and HB24 was found to be independent of protein synthesis implicating these genes as activation genes in lymphocytes. In situ hybridization studies have revealed expression of these genes in lymphoid and in certain developing tissues.

We have also begun to characterize genomic clones for CD22, HB9, HB24, and CD20. Emphasis has been on characterizing the promoter regions for each of these genes and on providing probes for chromosomal localization. The CD20 promoter has been most extensively characterized and we have begun to identify the cis sequences important in the B cell specific expression of CD20. (Deguchi, Rieckmann, Fauci, Kehrl, LIR/NIAID).

Regulation of Human B Cell Function by Soluble Factors

We have continued our studies of the role of soluble factors in the regulation of human B cell proliferation and differentiation. Our recent studies have focused on two autocrine factors produced by B cells, TNF-α and IL-6. Normal tonsillar B cells were found to synthesize and secrete low levels of both TNF-α and IL-6; however, *in vitro* B cell activation resulted in a dramatic increase in the synthesis and secretion of both factors. The kinetics of production suggested that the secretion of TNF-α was important in the induction of IL-6 production and an antibody to TNF-α inhibited B cell IL-6 production. Furthermore, *in vivo* activated B cells were also found to synthesize and secrete TNF-α and IL-6. The addition of an IL-6 antibody to B cell cultures inhibited the synthesis of Ig by both *in vivo* and *in vitro* activated B cells. These studies further demonstrate the importance of autocrine production of TNF-α and IL-6 in human B cell function. Current studies are focusing on the interactions between these factors and TGF-β in the regulation of B cell function and on determining whether CD5 positive B cells are important in cytokine production (Rieckmann, Fauci, Kehrl, LIR/NIAID).

Effect of TGF-β on Lymphocyte Function

Collaborative studies have demonstrated the overproduction of TGF-β by leukemic T cells obtained from patients with adult T cell leukemia. The increase in TGF-β production by these cells may be related to the HTLV-1 p40x (TAX) protein which we have shown transactivates the TGF-β1 promoter. The cis element in the TGF-β promoter was mapped to an AP-1 site. We have also shown that other isoforms of TGF-β inhibit immune function

including human TGF-β2, xenopus TGF-β2 and xenopus TGF-β5. We have completed other studies on the inhibitory effects of TGF-β on B cell Ig synthesis and secretion. TGF-β was shown to dramatically decrease steady state kappa and lambda light chain mRNA levels. This decrease was not related to an inhibition of Oct-2, NF-κB or kE2 binding factors as assessed by mobility shift assays. TGF-β was found to decrease AP-1 both in normal *in vitro* activated B cells and certain B cell lines. The B cell line, HS-Sultan, is responsive to TGF-β and TGF-β inhibits both AP-1 and kappa light chain in this cell line similar to normal B cells. This cell line will be valuable for transfection studies with kappa promoter and enhancer constructs in order to map the cis elements important in the inhibition of Ig synthesis by TGF-β. (Thevenin, Fauci, Kehrl, LIR, NIAID).

Production of Endothelins by Human Monocytes/Macrophages

Endothelins are peptides originally isolated from endothelial cells with potent vasoactive and mitogenic properties. We have demonstrated that human macrophages synthesize and secrete endothelins by both immunochemistry and a combination of reverse-phase high performance liquid chromatography (HPLC) with radioimmunoassays specific for either endothelin-1 or -3. The secretion of endothelin-1 but not of endothelin-3 by macrophages could be stimulated 6 to 10 fold by lipopolysaccharide (LPS) or PMA. No immunoreactive endothelin was detected in cell extracts from human neutrophils or lymphocytes. The expression of endothelins in tissue macrophages was demonstrated in paraffin sections of human lung using immunohistochemistry. Macrophage derived endothelins may have an essential function in blood vessel physiology and aberrant production may contribute to vessel pathology. Current studies are examining the potential role of endothelins in the pathogenesis of diseases characterized by vessel pathology (Ehrenreich, Fox, Rieckmann, Hoffman, Kehrl, Fauci, LIR/NIAID, Anderson, Coligan, BBR/NIAID)

The Genetic Response of Primary T Lymphocytes to an Activation Signal: Control of Proliferation and Immune Competence

We have characterized a number of selected novel genes from the collection of rapidly inducible cDNA clones isolated from mitogenically stimulated human T cells. These genes are likely to control the cascade of events which culminate in proliferation and expression of a differentiated T cell phenotype. Among the novel genes is a zinc finger containing DNA binding protein which is also expressed in all HTLV-I or HTLV-II transformed cells analyzed to date. T cell transformation by this virus may be mediated by cellular regulatory genes such as the zinc finger gene to effect proliferation. In addition, we are studying genes encoding a membrane antigen protein which is expressed only in cells of hematopoietic origin and a rel oncogene related protein, which may be an important transactivator of other cellular genes. We have identified the chromosomal location of two previously isolated novel lymphokine/cytokine genes which coincides with the loci for two diseases, von Recklinghausen neurofibromatosis and chronic myelogenous leukemia. Also, we have demonstrated that these two proteins are among a large collection of factors which are activated in bone-marrow derived mast cells by several different signalling mechanisms. As part of our detailed studies on the regulation of inducible genes we have shown that the activation-dependent binding of the NF-κB nuclear complex to the HIV enhancer can be sensitive to the immunosuppressive drug cyclosporin A, depending on the activation signal used. (Siebenlist, Burd, Wright, Bours, LIR/NIAID; Costa, Metcalfe, LCI/NIAID; Kelly, LP/LCI; Rapp, FCRF/NCI; Greene, Duke Univ. Med. School; Fleckenstein, Erlangen Univ., FRG; Morton, Harvard Univ., Henninghausen, LBM/NIDDK).

Clinical, Immunopathogenic, and Therapeutic Studies in the Vasculitides and Other Immune-Mediated Diseases

Over the past year we have expanded our studies of the clinical, immunopathogenic, and therapeutic aspects of the entire spectrum of vasculitis. We have continued studies demonstrating remission and cure rates in systemic vasculitis using the combination of daily cyclophosphamide and prednisone. Concerns regarding the longterm toxicities of daily cyclophosphamide have led to the evaluation of alternative treatment protocols. We have demonstrated that intermittent high dose intravenous cyclophosphamide is less effective than daily low dose oral cyclophosphamide in the treatment of Wegener's granulomatosis. We have preliminary data indicating that weekly methotrexate may be effective in the treatment of Takayasu's arteritis. We are currently evaluating the effectiveness of methotrexate in the treatment of all types of systemic vasculitis. In addition, studies are ongoing to evaluate the use of trimethoprim/sulfamethoxazole therapy for Wegener's granulomatosis. Studies of the use of bronchoalveolar lavage as a tool to study pathophysiology of Wegener's granulomatosis have demonstrated that active disease is associated with a neutrophilic alveolitis. These studies have also documented in situ pulmonary production of antineutrophil cytoplasmic antibodies. (Hoffman, Leavitt, Fauci, LIR/NIAID; Travis, LP/NCI; Fleischer, DCP/CC).

Immunopathogenesis of Chlamydia Trachomatis Infections

During the past year our laboratory has continued its studies on examining the transmission efficiency of chlamydial infections in association with other genital infections such as gonorrhea and human papillomavirus. It is evident that chlamydia is a "prevalent" disease in that infection rates vary very little by either sex, reason for clinic attendance, number of sex partners or the nature of patients' relationship with their sexual partners. In contrast, gonorrhea has the characteristics of an "incident" disease occurring more often in men with more sexual partners, those involved in new or casual relationships, and in men who attend STD clinics for symptom evaluation. By studying couples prospectively we can carefully assess the relationship of these two infections in transmission and examine the role of host immunity in chlamydial genital infections. We have demonstrated that the most common chlamydia serovars are D (35%) and F (48%), and that serovar F appears to induce or maintain an inflammatory response in urethritis ($p=0.027$) in contrast to the other serovars. Furthermore, the high prevalence of antichlamydial antibodies was noted in 65% of the men and 94% of the women, and that women (39%) had higher titers (>1:128) of chlamydial specific antibody than men (15%). The PCR for detection of chlamydial DNA was further refined and modified with the adaptation of soluble hybridization with an RNA probe producing RNA-DNA hybrids which could be quantified in an EIA. This format of the PCR was shown to have a sensitivity and specificity of 98.5% compared to culture and was capable of detecting one IFU/ml. In an experimental animal model system where the infection can be controlled and monitored, we have found that chlamydial DNA can be detected following repeat inoculations despite negative cultures suggesting the persistence of chlamydia in a latent or cryptic form. (Quinn, LIR/NIAID).

Honors, Awards, and Scientific Recognition

Over the past year, members of the LIR have received a number of awards and honors.

Dr. Fauci continues to serve on a number of committees of scientific and administrative importance such as the U.S. Public Health Service AIDS Executive Task Force and as chairman of the National Institutes of Health AIDS Executive Task Force.

Dr. Fauci and other members of the LIR serve on editorial boards of journals concerned with the areas of immunology, allergy, and infectious diseases. This past year, Dr. Fauci became a member of the Editorial Advisory Board of Immunopharmacology and Immunotoxocology. He continues his role as an Associate Editor for The American Journal of Medicine. He is also on the Editorial Board of The Journal of Immunopharmacology, Clinics in Immunology and Allergy, EOS, Annals of Allergy, Physicians' Journal Update, Cellular Immunology, Immunopharmacology, The Journal of Molecular and Cellular Immunology, AIDS Research and Human Retroviruses, and AIDS Patient Care. He serves on the Advisory Board of Clinical Immunology and Immunopathology and the Journal of Clinical Immunology. He is on the Editorial Advisory Council of La Ricerca in Clinica e in Laboratorio, the International Scientific Committee of Immunologia Clinica e Sperimentale, and is Consulting Editor for North America for the journal Thymus.

In the past year, Dr. Fauci became an editor of the "Transactions of the Association of American Physicians." He continues as an editor of "Harrison's Principles of Internal Medicine," "Current Therapy in Allergy, Immunology and Rheumatology," and "Harrison's Principles of Internal Medicine - Companion Handbook." He is an associate editor of "Current Therapy in Internal Medicine." In addition, he continues to edit with Dr. John I. Gallin "Advances in Host Defense Mechanisms."

Dr. Lane continues to serve on the editorial boards of AIDS Research in Human Retroviruses, Journal of the Acquired Immune Deficiency Syndromes, Journal of Clinical Immunology, and Clinical Immunology and Immunopathology. Dr. Quinn continues to serve on the editorial boards of AIDS, AIDS Research in Human Retroviruses, Journal of the Acquired Immune Deficiency Syndromes, The International Journal of STD and AIDS, and Sexually Transmitted Diseases. Dr. Hoffman became a member of the Editorial Board of the Journal of Hepatogastroenterology.

Dr. Fauci, as well as several members of the LIR, were asked to deliver major named or invited lectureships during the year. Dr. Fauci was the Walter K. Myers Visiting Professor at the Washington Hospital Center. He delivered the keynote address at the Annual Meeting of the American Association of Physicians for Human Rights. He was an invited lecturer at the Marine Biological Laboratory at Woods Hole (Massachusetts) Friday Night Lecture Series. Dr. Fauci delivered plenary lectures at the Annual Meeting of the Infectious Diseases Society of America, the Annual Meeting of the Institute of Medicine, the Second (Republic of) San Marino Conference, the Fourth Meeting of "Cent Gardes" in Paris, the Annual New York/Italy Medical Symposium, the Annual Meeting of the American Association for the Advancement of Science, the Annual Meeting of the Association of American Physicians, and the VIth International Conference on AIDS.

He was an invited plenary speaker at the National AIDS Update, the Second International Conference on Drug Research in Immunologic and Infectious Diseases, the

Annual Meeting of the American Federation for Clinical Research, and the UCLA-Hoffman LaRoche-Smith, Kline & French Symposium - HIV and AIDS: Pathogenesis, Therapy and Vaccine. He was an invited symposium speaker at the Annual Meeting of the Clinical Immunology Society and the American Association of Immunologists Symposium on Contributions of Basic Immunology to Human Health as well as an invited speaker at the Brookings Institution Seminar Series on Risk Analysis. He was also an invited seminar speaker at the Public Health Research Institute in New York City, and an invited panel discussant at the annual meeting of the National Association of Social Workers.

In addition, Dr. Fauci delivered the Third Annual Farr Lecture of the Yale University School of Medicine, the Maloney-Booker Graduate Lecture on Pharmacology of the Howard University School of Medicine, and the Alpha Omega Alpha Lecture at Tufts University Medical School and at Cornell University Medical School. Of particular note, Dr. Fauci delivered the Donald F. Richardson Memorial Lecture of the American College of Obstetricians and Gynecologists, the Third Annual Martin V. Bonventre, M.D. Memorial Lecture of the Brooklyn Hospital, and the prestigious Seventeenth Annual Maxwell Finland Lecture of the Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School.

Finally, Dr. Fauci delivered the commencement address to The Graduate School and School of Medicine of Hahnemann University and to Harvard Medical School.

Dr. Lane was an invited symposium speaker at the VIth International Conference on AIDS and a visiting professor at Mt. Sinai School of Medicine.

Dr. Quinn delivered the Lester Breslow Distinguished Lectureship in Epidemiology and the John F. Kessel Distinguished Lectureship in Tropical Medicine at the University of California, Los Angeles.

Dr. Koenig served as a consultant to the American Medical Association. He also was an invited speaker at the Second (Republic of) San Marino Conference and the Annual Meeting of the American Association of Immunologists.

Dr. Poli delivered invited lectures at the Annual Meetings of the Association of American Physicians and the Federation of American Societies for Experimental Biology, and at the UCLA Symposium on Molecular Pathways of Cytokine Action, a Symposium on the Pathogenesis of HIV Infection, and the III Convegno Nazionale, AIDS e Sindromi Correlate, in Naples, Italy.

Over the past year Dr. Fauci received several awards including the 1989 Achievement Award of the American Association of Physicians for Human Rights, the 1989 National Medical Research Award of the National Health Council, the Flame of Hope Award of the Terri Gotthelf Lupus Research Institute, the 1989 Maxwell Finland Award in Infectious Disease from the National Foundation for Infectious Diseases, the 1989 Helen Hayes Award for Medical Research, the Excellence in Public Service Award of the Committee for the Support of Public Service, and the 1990 Lifetime Science Award of the Institute for Advanced Studies in Immunology and Aging. Dr. Fauci also received the Doctor of Science, Honoris Causa, degree from Mount Sinai School of Medicine, Georgetown University School of Medicine, Hahnemann University Graduate School and School of Medicine, and Neumann College, Aston, PA.

Dr. Quinn received the U.S. Public Health Service Outstanding Service Award for International AIDS Research and a U.S.P.H.S. Outstanding Unit Citation for his work on sexually transmitted diseases. He also received the Winthrop Pharmaceutical Research Award and a commemorative award for international AIDS research from the University of Florida, Gainesville. Dr. Lane received the U.S.P.H.S. Outstanding Service Medal. Dr. Schnittman received the Young Investigators Award from the Interscience Conference on Antimicrobial Agents and Chemotherapy.

Administrative, Organizational, and Other Changes

The major theme of the LIR continues to be the delineation of the cellular and molecular mechanisms of regulation of the human immune response in normal and disease states. The vast majority of the activities of the LIR currently focus on AIDS, particularly the immunopathogenesis of HIV infection. There has been modest growth of the clinical AIDS unit of LIR in keeping with the Congressional mandate to expand these activities.

In the Immunopathogenesis Section of LIR, Dr. Andrew Dayton's unit is now fully functional and includes Dr. Elaye Dayton and graduate student Douglas Powell. Dr. Guido Poli, now a Visiting Associate will remain in LIR for the indefinite future. Dr. Giuseppe Pantaleo will also remain for at least 1 to 2 years. Drs. DeMaria and Butini will return to Italy by the end of the 1991 fiscal year. Dr. Sharilyn Stanley will continue on as a Senior Staff Fellow. Drs. Steven Schnittman and Scott Koenig are currently on a tenure track. Dr. Peter Bressler will continue in his present position. Drs. Cecilia Graziosa and Priscilla Biswas from Rome and Florence respectively have joined the LIR this year for post-doctoral fellowships. Dr. Tea Kalebic will be leaving LIR this year. Dr. Jin-Xin Hong is visiting from Shanghai and will remain in the LIR for 1 year. Dr. Hannelore Ehrenreich from Munich will continue on for an additional year as a Guest Researcher.

In the Clinical and Molecular Retrovirology Section, Dr. Y. Tani was promoted to a Visiting Associate. Dr. John Zurlo has left LIR to join the faculty at Pennsylvania State University at Hershey, PA. Dr. K. Zunich has left to join the DAIDS of NIAID. Dr. R. Walker will return to LIR to rejoin the Clinical AIDS Unit. Ms. Diane Lee has left to pursue other career opportunities. The two new Medical Staff Fellows who have joined the LIR are Drs. Drew Weismann and Lawrence Fox, both of whom will be doing clinical rotations this year.

Finally, both Drs. Thomas Quinn and John Kehrl were granted tenure this year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00537-03 LIR

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunopathogenic Mechanisms of Human Immunodeficiency Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.S. Fauci	Chief	LIR, NIAID
Others:	S. Schnittman	Senior Staff Fellow	LIR, NIAID
	G. Poli	Visiting Associate	LIR, NIAID
	P. Bressler	Senior Staff Fellow	LIR, NIAID
	S. Stanley	Medical Staff Fellow	LIR, NIAID
	G. Pantaleo	Visiting Associate	LIR, NIAID

COOPERATING UNITS *(if any)*

LMM, NIAID, (A. Rabson, W. Maury, M. Martin); PRI, Fredrick, MD, (M. Baseler); Duke Univ. Med. Center, (B. F. Haynes, S. Denning, J. Kurtzberg); Dept. Pathol., George Washington Univ., (J. Orenstein); LVD, NIAID, (B. Moss); LTCB, NCI, (S. Colombini, P. Lusso); Dept. Biochem.,

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
17	13	4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is directed at delineating the cellular and molecular mechanisms of the immunopathogenesis of HIV infection. Using the chronically infected T cell (ACH-2) and promonocytic (U1) cell lines we have made a number of observations. The endogenous cytokines tumor necrosis factor alpha (TNF-α) (in ACH-2) and TNF-α, interleukin (IL)-6, and granulocyte-macrophage colony stimulating factor (GM-CSF) (in U1) can potently upregulate HIV expression. TNF-α induces HIV expression in an autocrine and paracrine manner, while IL-6, GM-CSF, and TNF-α synergize in HIV induction in U1 cells. TNF-α acts by inducing new transcription via an NF-kB mechanism, while IL-6 and GM-CSF either alone or in combination act by post-transcriptional mechanisms. The pleotropic cytokine transforming growth factor-beta (TGF-β) downregulates the induction of HIV by phorbol myristate acetate (PMA), IL-6, and GM-CSF, but not by TNF-α. Glucocorticoids (GC) are post-transcriptional synergistic co-inducers with IL-6, GM-CSF, and interferon (IFN)-gamma of HIV expression in U1 cells. Heat shock (42°C) alone induces HIV expression by increasing transcription from the HIV-LTR and physiologic heat (40°C) synergizes with IL-6, GM-CSF, or GC in the induction of HIV expression. Activated B cells from HIV-infected individuals constitutively produce increased amounts of TNF-α and IL-6 and are capable of inducing HIV expression in U1 and ACH-2 cells in co-culture or via supernatants. We have shown that the reservoir of HIV infection in the peripheral blood is the CD4+ T cell. Also, there is a direct correlation between viral burden, depletion of CD4+ T cells and disease progression. HIV preferentially infects the memory subset of CD4+ T cells. HIV infects intrathymic T cell precursors which may explain the inability to completely regenerate CD4+ T cells even during effective anti-retroviral therapy. There is a progressive and preferential loss during the course of HIV infection of HIV-specific CD8+ cytolytic function as opposed to broad cytolytic potential. We have demonstrated that CD8+ T cells can be infected with HIV in vitro by direct contact with infected CD4+ cells. We have also demonstrated the presence of in vivo infected CD8+ cells. Leukocyte adhesion molecules, particularly LFA-1 are involved in the cell to cell spread of HIV, and this spread can be blocked by antibodies to these molecules.

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Others:	A. De Maria	Guest Researcher	LIR, NIAID
	L. Butini	Guest Researcher	LIR, NIAID
	H.C. Lane	Senior Investigator	LIR, NIAID
	S. Koenig	Senior Staff Fellow	LIR, NIAID
	C. Fox	Senior Scientist	LIR, NIAID
	J. Kehrl	Senior Investigator	LIR, NIAID
	P. Rieckmann	Guest Researcher	LIR, NIAID
	P. Biswas	Guest Researcher	LIR, NIAID
	T. Kalebic	Visiting Associate	LIR, NIAID
	C. Graziosa	Visiting Associate	LIR, NIAID
	C. Thevenin	Guest Researcher	LIR, NIAID

Cooperating Units: Cornell University Medical Center (A. Meister, M. Anderson); Lab. Chemoprevention, NCI, (M. Sporn); Georgetown Univ., (M. Psallidopoulos, N. Salzman, B. Fernie, P. Pierce); NMRI, (S. Kessler); COB, NCI (H. Mitsuya); PB, NCI (P. Pizzo); Harvard Med. School (H. Winter); Center for Blood Research, Boston (T. Springer); St. Luke's Hospital, NY (D. Kotler); Mama Yemo Hospital, Kinshasa, Zaire (Kapita Bila, Musengala); Dept. Mol. Genetics and Microbiology, UMDNJ, Piscataway, NJ (S. Petska).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00585-01 LIR

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathogenic Mechanisms in Human Immunodeficiency Virus and Other Retroviral Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H.C. Lane	Senior Investigator	LIR,NIAID
Others:	D.I. Cohen	Expert	LIR,NIAID
	Y. Tani	Visiting Associate	LIR,NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Immunoregulation

SECTION
Clinical and Molecular Retrovirology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 4.5	PROFESSIONAL: 2.5	OTHER 2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to dissect the molecular mechanisms whereby HIV-1 functionally alters or kills CD4+ T lymphocytes a series of transfected Jurkat cell lines expressing different HIV-1 proteins were established. Cells constitutively expressing functional gp120 and gp41 had no alterations in cell growth nor in their ability to support the growth of HIV-1. Cells expressing HIV-1 nef appeared normal in expressing IL-2 and in supporting HIV-1 replication. Transfection of Jurkat T cells with HIV-1 envelope in defined situations resulted in the production of truncated p75 envelope- related protein. HIV-1 infection of a variety of CD4+ cells or tumors led to increases in two tyrosine-phosphorylated proteins of 95 and 40 kilodaltons while phosphoproteins pp56 and pp59, likely corresponding to p56 lck and p59fyn, were unaltered. Peripheral blood CD4+ T lymphocytes infected in vitro with HIV-1 were found to give rise to CD4-/CD8- gamma/delta T lymphcytes that did not express IL-2 following stimulation. In studies on the peripheral blood mononuclear cells of patients with HIV infection an increased proportion of cells expressing the gamma/delta T cell receptor were identified. Two variable region immunoglobulin genes encoding human antibodies directed against gp41 were cloned.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00587-01 LIR

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Gene Expression of the Human Immunodeficiency Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.I.Dayton	Sr. Staff Fellow	LIR,NIAID
Others:	E.T. Dayton	Staff Fellow	LIR,NIAID
	D.M.Powell	Graduate Student	LIR,NIAID
	T.Jeang	Senior Staff Fellow	LMM,NIAID

COOPERATING UNITS *(if any)*

LTCB, NCI, (M. Klotman); LTCB, NCI, (S. Daefler); Wistar Inst., Philadelphia, PA, (K.Nishikura); J. N. Gamble Inst., Cincinnati, OH (B. Baroudy)

LAB/BRANCH
Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 3	PROFESSIONAL: 2	OTHER: 1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We determined the structure of CAR, the RNA target sequence of the rev protein of HIV-1 and identified which of the sub-structures of CAR are essential for function. Site-directed mutagenesis was employed to alter selected sequences in CAR enabling us to determine 14 regions which basepaired to form 7 stem structures. By computer analyses we will then predict with confidence the secondary structure of the entire sequence. We have continued this analysis and have accumulated evidence for limited sequence specificity in the interaction between CAR and viral and/or cellular binding proteins. Using band shift analysis we have studied the binding of REV to CAR and have tentatively identified regions which bind REV and regions which probably bind cellular factors. We have also initiated studies on the interaction of nuclear dsRNA unwindase (/adenosine deaminase) with CAR. In other work we have initiated studies on the possible interaction of the vif protein of HIV-1 with other viral genes. HIV clones with vif mutations in different viral genetic backgrounds have different cellular tropisms which do not clearly mirror parental tropisms. We are presently exchanging sequences between two vif mutants with divergent tropisms to determine the sequences responsible for the differences.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00562-02 LIR

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Neuroimmunology and Neuropathogenesis of HIV Infection

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	L. Vitkovic	Expert	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	A. da Cunha	Visiting Associate	LIR, NIAID
	H. Ehrenreich	Guest Researcher	LIR, NIAID

COOPERATING UNITS (*if any*)
BRB, NIAID (J. Coligan); NINDS (M. Brightman, E. Major), NIDDK (Y. Sei); NCI (D. Katz); FDA (R. Anand); INSERM Unite 44, Strasbourg, France (M. Mersel, N. Neskovic)

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INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 3	PROFESSIONAL: 2	OTHER: 1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Human astrocytes were grown in culture and shown to secrete molecules which stimulated HIV-1 expression in a chronically infected promonocytic clone, U1.1.5. Interleukin-6, shown by others in the laboratory to synergize with TNF-α in stimulating HIV-1 expression in U1.1.5 cells, was detected in human and rat astrocyte-conditioned media. Transforming growth factor beta (TGF-β) was detected in herpes zoster virus-infected human brain and in human and rat astrocytes exposed to HIV-1 in vitro. Interleukin-1 was found to stimulate TGF-β expression in cultured rat astrocytes, microglia and oligodendrocytes. This stimulation was regulated post-transcriptionally in a time- and dose-dependent manner in primary rat astrocytes. These cells were also shown in vitro to secrete and bind endothelin-3 a potent vasoconstrictor. Exposure of rat granule neurons to HIV-1 resulted in their death. Brain isolate of HIV-1 BRVA, was the most potent isolate tested in the destruction of neurons and neurites. Taken together, these results suggest that both direct and indirect mechanisms may operate in the neuropathogenesis of HIV-1 infection and that the indirect mechanism may involve astrocytic cytokines.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00586-01 LIR

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immune Response to the Human Immunodeficiency Virus and Related Retroviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. Koenig	Senior Staff Fellow	LIR, NIAID
Others:	L.V. Wood	NRSA	LIR, NIAID
	A.S. Fauci	Chief	LIR, NIAID

COOPERATING UNITS *(if any)*

LVD, NIAID (B. Moss, P. Earl, V. Karacostas); NI,NINDS (S. Jacobson, D. McFarlin); Kyoto University (H. Shida); Molecular Vaccines, Inc. (T. Fuerst, R. Woods, A. Newell, A. Brewah).

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SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 4	PROFESSIONAL: 2	OTHER: 2

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our efforts have been concerned primarily with cell mediated immune responses to the two human retroviruses causing diseases in man, namely HTLV-1 and HIV. We have demonstrated that chronic infection with either virus may result in high levels of circulating cytotoxic T cells (CTL) that can be detected in freshly separated peripheral blood mononuclear cells (PBMC). These CTL are $CD8^+$ and MHC Class I restricted. In individuals infected with HIV, CTL activity is found more often in healthy patients with stable $CD4^+$ counts, particularly in the subset of these patients with detectable levels of p24 antigen in their sera. CTL activity appears to decline with clinical progression to AIDS. In contrast, in patients infected with HTLV-1, high levels of these circulating CTL are only found in patients with neurological disorders, particularly individuals with tropical spastic paraparesis (TSP). CTL in HIV-1 infected individuals are primarily generated against structural proteins, while in patients with TSP, the predominant response is against products of the pX region. Using limiting dilution cloning techniques and mitogenic stimulation, we have generated HIV-1 and HTLV-1 specific CTL clones. Using synthetic peptides, we have mapped two new CTL epitopes within HIV-1 gag and nef proteins. We have demonstrated that CTL epitopes to HIV-1 proteins can be mapped directly using freshly separated PBMC. We have also shown that a synthetic peptide containing the correct epitope may not be recognized by CTL due to surrounding amino acid sequences that may influence binding to HLA Class I and recognition by the T cell receptor. We have noted that CTL to nef can lyse HIV infected cells and such clones can inhibit HIV replication in vitro. We have found that if these clones are activated with peptides containing the appropriate epitope, inhibition of viral replication can occur through the release of soluble products from these cells, obviating the need for contact with $CD4^+$ cells harboring HIV. We have also defined a method for mapping B cell epitopes that are recognized by antibodies mediating antibody-dependent cellular cytotoxic responses (ADCC) and have shown that high levels of circulating $CD16^+$ cells are armed with these antibodies in vivo.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00390-07 LIR

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Investigation of the Acquired Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H.C. Lane	Senior Investigator	LIR,NIAID
Others:	A.S. Fauci	Chief	LIR,NIAID
	R.T. Davey	Medical Officer	LIR,NIAID
	K.M. Zunich	Medical Officer	LIR,NIAID
	J.J. Zurlo	Medical Officer	LIR,NIAID
	M. Easter	Nurse Practitioner	LIR,NIAID
	D. Lee	Project Coordinator	LIR,NIAID

COOPERATING UNITS (if any)

CC, NIH (H. Masur, J. Kovacs, J. Falloon, M. Polis, D. Henderson); NEI, NIH (R. Nussenblatt)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 7.5	PROFESSIONAL: 7.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An intensive effort was directed toward studying the epidemiologic, preventive and therapeutic aspects of the acquired immunodeficiency syndrome. An ongoing study of 1344 health care workers has continued to demonstrate the low risk of human immunodeficiency virus-1 (HIV-1) transmission from patient to health care worker with a seroconversion rate of 0.6% per percutaneous exposure. A 24 month follow-up of 48 high-risk individuals with indeterminate patterns on HIV Western blots failed to reveal a pattern indicative of early exposure and demonstrated a less than 3 month interval between polymerase chain reaction (PCR) positivity and full seroconversion. Immunization of healthy human volunteers to the gp160 envelope precursor protein of HIV-1 was capable of inducing group specific T lymphocytes directed toward the HIV-1 envelope and titers of neutralizing antibodies as high as 1:800. A placebo controlled trial of interferon-alpha in patients with early HIV-I infection demonstrated a decrease in the rate of progression for patients on interferon. Phase I trials of 3'-azido-2',3'-dideoxyuridine and rCD4-IgG failed to demonstrate efficacy in HIV infection as manifest by lack of significant change in surrogate markers. GM-CSF was found capable of reversing the neutropenia caused by the combination of interferon-alpha and ZDV. A clinical trial was initiated to compare the effects of ZDV to interferon-alpha to a combination of the two drugs in patients with early HIV-1 infection. A masked, randomized, delayed therapy controlled trial of foscarnet demonstrated this drug to be of benefit to patients with AIDS related CMV retinitis. Phase I/II trials were initiated of ZDV+IL-2, interferon-alpha+IL-2, DDI+interferon-alpha and BW566C80, the latter for the treatment of pneumocystis carinii pneumonia and toxoplasmosis. Immunotherapy protocols were initiated involving active immunization of HIV-1 infected individuals with HIV-1 gp160 or p24 and passive immunotherapy utilizing peripheral blood lymphocytes and bone marrow from gp160 primed donors.

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Others:	V. Davey	Project Coordinator	LIR,NIAID
	J. Metcalf	Biologist	LIR, NIAID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00361-08 LIR

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

International Studies on the Acquired Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.C. Quinn	Senior Investigator	LIR, NIAID
Other:	A.S. Fauci	Chief	LIR, NIAID
	C. Brown	Expert	LIR, NIAID

COOPERATING UNITS *(if any)*

CDC (R. Ryder); Institute of Tropical Medicine, Antwerp, Belgium (P. Piot, J. Periens); Johns Hopkins University (E. Hook, N. Halsey).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The acquired immunodeficiency syndrome (AIDS) has become a global pandemic with over 260,000 cases reported from 156 countries. A major focus within our laboratory has been on defining the unique epidemiologic, clinical, virologic and immunologic features of HIV-1 infection in Africa and other tropical countries. In a survey of 12,032 employees and their spouses at two large businesses in Kinshasa, Zaire, 4.5% were seropositive for HIV-1 with a 0.5% annual seroincidence predominately due to heterosexual transmission. In this study, AIDS continued to be the leading cause of death among employed individuals responsible for 50% of deaths. We also demonstrated that 40% of 1200 female prostitutes in Kinshasa were HIV-1 seropositive and that incident infections (3%/yr) were strongly correlated with genital ulcers and chlamydial infections. In studies of perinatal infection in Zaire, Kenya and Haiti, we documented a 25-35% transmission rate of HIV-1 infection and a cumulative mortality rate of 70% in the first two years of life. Of surviving children during the first year of life a beneficial effect of immunizations against commonly occuring childhood infectious diseases was observed despite the lower immunogenicity in HIV-1 seropositive individuals. In virologic studies we demonstrated the presence of HIV-1 genome by PCR and/or culture in 5% of 101 seronegative high risk individuals confirming a prolonged latency period before seroconversion. HIV-1 antigenemia was also observed to be decreased in Africans and U.S. blacks, probably secondary to p24 antibody-antigen complexes secondary to increased p24 antibody levels. Additional studies demonstrated the presence of neutralizing antibody to the V3 loop region of HIV-1_{mn} in HIV-1 infected individuals in the U.S., Zaire and Brazil suggesting the importance of this particular epitope of HIV-1_{mn}. Serologic screening for HTLV-I/II demonstrated increasing rates of HTLV-I infection among Africans and blood transfusion recipients in the U.S., and HTLV-II among IV drug users. Further studies will continue to examine the immunopathogenesis and natural history of these human retroviruses in selected populations.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00210-10 LIR

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunoregulation of Human Lymphocyte Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.H. Kehrl	Senior Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	C. Fox	Senior Scientist	LIR, NIAID
	P. Rieckmann	Guest Researcher	LIR, NIAID
	Y. Deguchi	Guest Researcher	LIR, NIAID
	C. Thevenin	Guest Researcher	LIR, NIAID
	H. Ehrenreich	Guest Researcher	LIR, NIAID

COOPERATING UNITS *(if any)*

BRB, NIAID (R. Anderson, J. Coligan); LCP, NCI (A. Roberts, S.J. Kim, M. Sporn); Metabolism Branch, NCI (J. Burton, R. Nordan); FDA (M. Norcross).

LAB/BRANCH
Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 5	PROFESSIONAL: 4	OTHER: 1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued our studies of the mechanisms of B cell activation, proliferation, and differentiation at the molecular and cellular levels. We have isolated a cDNA clone for the B cell membrane antigen CD22 and have shown that it functions to mediate B-B cell interactions and may be in part responsible for the maintenance of B cell enriched regions in lymphoid tissues. It is referred to as BL-CAM an acronym for B lymphocyte cell adhesion molecule. The CD20 promoter has been sequenced and CAT constructs spanning 4 kb 5 prime to the transcriptional start site have been made. These CAT constructs have been used to transfect B and T cell lines and a region necessary for B cell specific expression has been mapped. Current studies are devoted to determining the precise cis element(s) necessary for B cell specific expression. We have isolated 3 homeotic genes which are expressed in activated B cells. One of the genes, HB24, encodes for a protein with a homeobox highly related to a drosophila homeodomain, H2. These genes are likely to have important regulatory roles in certain developing tissues and in lymphocytes. Both in vivo and in vitro activated B cells have been found to secrete TNF-α and IL-6 and their production has been shown to be important in B cell function. TGF-β has been shown to markedly inhibit the production of kappa and lambda light chain in B cells. The decrease in light chain mRNA is not accompanied by changes in 3 transcriptional factors (Oct-2, NF-kB, and kE2 binding proteins) known to be important in kappa transcription. TGF-β did decrease AP-1 levels both in normal B cells and in B cell lines. We have begun to study the role of phosphatases in B and T cell activation and have found okadaic acid, a specific phosphatase inhibitor, to be a potent activator of NF-kB, AP-1, and TNF-α mRNA (only B cells). Studies in collaboration with the metabolism branch and the Laboratory of Chemoprevention have demonstrated an increased production of TGF-β by leukemic cells from patients with adult T cell leukemia. The increase in TGF-β may be related to the transactivation of the TGF-β promoter by the HTLV-1 p40x (TAX) protein. The cis response element in the TGF-β promoter was mapped to two AP-1 sites known to be important in the autoinduction of TGF-β. Finally we have demonstrated the production of potent vasoactive peptides, endothelin-1 and -3, by human macrophages and monocytes. Macrophage derived endothelins may be important regulatory factors which function within the macrophage microenvironment.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00431-06 LIR

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Molecular Biologic Approach to Immune Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	U. Siebenlist	Senior Staff Fellow	LIR, NIAID
Others:	P. Burd	Natl. Res. Serv. Fellow	LIR, NIAID
	J. Wright	Guest Researcher	LIR, NIAID
	V. Bours	Guest Researcher	LIR, NIAID
	A.S. Fauci	Chief	LIR, NIAID
	J. Costa	Medical Staff Fellow	LCI, NIAID
	D. Metcalfe	Section Chief	LCI, NIAID

COOPERATING UNITS *(if any)*
LP/NCI (Kelly); FCRF/NCI (Rapp); DCBD/NCI (McBride); Duke Univ. Med. School (Greene); Erlangen University, W. Germany (Fleckenstein); Harvard University (Morton); LBM/NIDDK (Hennighausen).

LAB/BRANCH
Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 5.5	PROFESSIONAL: 4.0	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have studied the primary genetic response of human peripheral blood T cells to mitogenic activation. By employing subtractive cloning technology, we have previously isolated in excess of 69 genes whose expression is induced within hours of cellular stimulation. Included among these genes are well-known lymphokines, receptors and oncogenes in addition to many novel genes whose functions are suspected to be similarly important for proliferation and the expression of a differentiated T cell phenotype. We have pursued both functional as well as regulatory aspects of several selected novel genes. Two genes encode novel lymphokines which belong to a family of pro-inflammatory factors. We have shown that these genes are among the many cytokines which are inducible by IgE in mast cells. We have localized the lymphokine genes to chromosome 17q, near the loci for von Recklinghausen neurofibromatosis and for acute promyeolocytic leukemia. The two genes are closely linked in the genome in a head to head fashion and we are studying their promoter/enhancer elements. We have done similar studies on the IL-2 and HIV enhancers, which serve as our model systems. We have discovered that an NF-KappaB complex interacting with these enhancers can be activated via cyclosporin A sensitive and insensitive pathways. Three further genes selected for analysis are DNA binding regulatory factors, one of which belongs to the family of steroid receptors and the other two are zinc finger domain containing nuclear proteins. One of the zinc finger proteins is constitutively expressed in all HTLV I or HTLV II transformed cells analysed to date. We have demonstrated that this gene is transcriptionally induced by the HTLV I tax product and that it is expressed constitutively also in T cells transformed with a herpesvirus saimiri vector carrying tax. Several additional induced and novel genes are under intense investigation, including one encoding a membrane protein of the class II receptor family and another gene with extensive N-terminal homology to the c-rel oncogene and the homeotic gene dorsal of Drosophila.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00213-10 LIR

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies in the Vasculitides and Other Immune-Mediated Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A.S. Fauci	Chief	LIR, NIAID
Others:	G.S. Hoffman	Senior Investigator	LIR, NIAID
	R.Y. Leavitt	Senior Investigator	LIR, NIAID
	G.S. Kerr	Medical Staff Fellow	LIR, NIAID

COOPERATING UNITS (if any)

NIAID, NIH, (J.M.G. Sechler); NIAID, NIH, (J.I. Gallin); CC, NIH, (J.H. Shelhamer); CC, NIH, (A. Suffredini); CC, NIH, (F.P. Ognibene); NCI, NIH, (R.J. Baltaro); NCI, NIH, (T.A. Fleisher); NCI, NIH, (W.D. Travis); NIAID, NIH, (S.E. Straus); LDBA, NIH, (H. Kleinman); LDBA, NIH, (D. Grant); CC,

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have continued to study the efficacy of daily low dose cyclophosphamide and prednisone as remission-inducing and potentially curative therapy in systemic vasculitis. The long-term toxicity of daily cyclophosphamide therapy in some patients has led to studies of alternative treatment including (1) intermittent intravenous high dose cyclophosphamide, and (2) weekly low dose methotrexate in each of the systemic vasculitides, and (3) daily trimethoprim/sulfamethoxazole in limited Wegener's granulomatosis. A prospective analysis of bronchoalveolar lavage in Wegener's granulomatosis and controls has demonstrated that disease exacerbations are characterized by neutrophilic alveolitis, macrophage-leukocyte phagocytosis and the production of anti-neutrophil cytoplasmic antibodies in the lung. These observations suggest that neutrophilic inflammation and abnormal immune reactivity to neutrophil antigens may play a role in the pathogenesis of this disease. The pathogenesis of the vasculitides is also being investigated in vitro with the aid of endothelial cell systems to (1) characterize leukocyte binding to normal control and autologous endothelial cells, (2) assess antibody reactivity and serum cytotoxicity to normal and autologous endothelial cells, and (3) determine whether sex hormones influence leukocyte-endothelial cell adhesion, a question that is particularly relevant to Takayasu's arteritis. An epidemiologic analysis of patients with Wegener's granulomatosis has continued to evaluate environmental factors, such as inhalant exposures and patient clusters.

PHS 6040 (Rev. 1/84) GPO 914-918

Cooperating Units: NIH, (J.R. Minor); NIAID, NIH, (M. Cid); NIAID, NIH, (C. Hallahan); DMID, NIH, (M.F. Cotch); DMID, NIH, (R.A. Kaslow); George Washington Med. Ctr., (J. Giordano) Laboratory of Immunoregulation NIAID, NIH, Bethesda, Maryland 20892

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00358-07 LIR

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunopathogenesis of *Chlamydia trachomatis* infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.C. Quinn	Senior Investigator	LIR, NIAID

COOPERATING UNITS *(if any)*

Johns Hopkins University, (E. Hook, S. Holland)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen in the US with an estimated 5 million cases annually. Studies have been in progress to define the clinical spectrum of chlamydial infection, to develop rapid diagnostic assays and to examine the pathogenesis of chlamydial infection in experimental animal models. In screening over 2000 men and women attending STD clinics, chlamydia genital infections continue to be common with infection rates of 10%-15%. Chlamydia infections tend to have the characteristics of a prevalent infection since infection rates vary little by either sex, reason for clinic attendance, number of sex partners or the patient's relationship with their sexual partners. The predominate serovars present in this population are serovar D (34.7%) and serovar F (47.8%), with serovar F more likely to induce or maintain an inflammatory response in urethritis ($p = 0.027$). A relatively high prevalence of anti-chlamydia antibodies was noted in 65% of the men and 94% of the women, and higher titers of chlamydia specific antibodies ($\geq$ 1:128) were more common among women (39%) than in men (15%). Patients with recurrent gonorrhea were significantly more likely to experience recurrence of chlamydia due to the same serovar, suggesting that microbiologically inapparent or persistent chlamydial infections may be reactivated during recurrent gonococcal infection. Diagnostic assays including antigen detection by solid phase EIA and polymerase chain reaction (PCR) have been developed. By incorporating soluble hybridization with an RNA probe and a fluorometric EIA to detect RNA-DNA hybrids, we have been able to quantitate the amount of DNA amplified by PCR. In an experimental animal model where viability of C. trachomatis can be monitored, we have demonstrated persistence of chlamydial DNA by PCR in monkeys who have become culture negative. This persistence of chlamydial DNA may possibly contribute to either a sustained hypersensitivity response or a latent state which is inhibited by the presence of neutralizing antibody and cell - mediated immunity. Further studies are planned to examine the persistence of chlamydia in patients to further define the immunopathogenesis of chlamydia and its capability for inducing latent infection. Additional studies are also planned to examine the prevalence and clinical presentation of C. pneumoniae (TWAR) infection as an etiologic cause of pneumonia in pediatric, adult and geriatric populations.

LABORATORY OF INFECTIOUS DISEASES
1990 Annual Report
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Summary Statement
LABORATORY OF INFECTIOUS DISEASES
National Institute of Allergy and Infectious Diseases
October 1, 1989 to September 30, 1990

INTRODUCTION

During FY1989 LID scientists continued to pursue the laboratory's long term goals of: (a) delineating the etiology, pathogenesis and epidemiology of medically important viral diseases, (b) defining the fine structure, genome organization and antigenic composition of these viral pathogens, and (c) using the techniques of molecular biology and immunology to develop successful strategies for treating or preventing these diseases. Considerable success was achieved in each of these areas of research during the past year.

HEPATITIS AND ANIMAL AIDS VIRUSES

Hepatitis A virus (HAV). HAV is a picornavirus which has a single strand, positive sense RNA genome of 7478 nucleotides that codes for a single long polyprotein. Wild type HAV grows very slowly and to low titer in cell culture. A cell culture adapted HAV mutant was selected after serial passage in primary simian kidney cell culture and shown to be attenuated for marmosets and chimpanzees. Full-length DNA clones were prepared from the wild type virus and its tissue culture mutant, but only full-length RNA transcripts of cloned mutant DNA were infectious for cell culture. Recently, the lack of infectivity of full-length RNA transcripts of cloned wild type HAV DNA was traced to a single base deletion in the middle of the DNA clone that caused a frame shift in the polyprotein sequence. Subsequently, the deletion was corrected by oligonucleotide directed mutagenesis yielding a DNA clone whose RNA transcripts were infectious for cell culture when the 5' non-coding region of the cloned mutant DNA was substituted for the corresponding wild type sequence. In contrast, a chimera that was identical except for the single base deletion was not infectious. The importance of the 734 nucleotide 5' non-coding region of the viral genome in replication *in vitro* emerged from an analysis of the growth of chimeric HAV viruses differing only in this region. Specifically, chimeric viruses containing the 5' wild-type sequence were restricted in their replication in the simian CV-1 cell line compared to viruses containing the 5' sequence of the cell-culture adapted mutant, whereas both types of chimeric virus grew efficiently in fetal rhesus kidney cell culture. This indicated that mutations in this region can impart a host range effect. Previously the 2B/2C non-structural protein region of the viral genome was shown to play a major role in determining replicative capacity in cell culture. Thus, two separate regions of the HAV genome contain important determinants of replicative capacity *in vitro*.

Greater than 95% of the sequence of a simian isolate of HAV was determined by direct sequencing of the polymerase chain reaction product obtained using specific primers to amplify RNA from a liver homogenate of an infected African green monkey. The sequence differs substantially from that of any known isolate of HAV. It is of interest that this virus grows very efficiently *in vitro* in both primary African green monkey kidney cells and a continuous cell line of fetal rhesus kidney cells.

Attempts were made to increase the efficiency of replication of HAV in tissue culture. A continuous simian cell line (fetal rhesus kidney) stably transformed with a plasmid containing

the P2 region (that encodes non-structural proteins 2A, 2B and 2C) of the Sabin 1 strain of poliovirus was shown to support the growth of the tissue culture adapted mutant of HAV. Significantly, the infectivity of the tissue culture-adapted HAV mutant was increased 10 to 100-fold when assayed in the poliovirus P2 transformed cell line compared to the non-transfected parental cell line. This suggests that complementation involving non-structural proteins of the P2 region may occur between picornaviruses as disparate as HAV and poliovirus. The poliovirus P2 transformed cell line may prove useful in increasing the efficiency of *in vitro* replication of wild type HAV that until now has grown very slowly and to low titer in the most permissive cell cultures available.

Hepatitis E virus (HEV). In 1980 LID scientists described a new form of viral hepatitis caused by a previously unrecognized hepatitis virus that is not related to HAV but nonetheless shares the epidemiologic characteristics of HAV, i.e., the newly identified virus is transmitted by the fecal-oral route, produces water-borne epidemics and is probably responsible for a significant proportion of endemic hepatitis. The disease caused by this virus has been called epidemic or enterically transmitted non-A, non-B hepatitis or hepatitis E. Such hepatitis is both epidemic and endemic throughout the Indian subcontinent, central Asia, parts of the Middle East and northern Africa, with probable extension to West Africa. Epidemics of the disease have also occurred in Mexico. The putative etiologic agent, a 27-30nm nonenveloped virus, was identified in a volunteer study in 1983 and similar virus-like particles have been detected in feces of cases from a number of other outbreaks. The small quantity of these virus-like particles available for study has limited progress but it is now possible to biologically amplify the virus by collecting bile from experimentally infected cynomologous monkeys. These monkeys as well as chimpanzees develop an elevation of liver enzymes 28 days post infection. Efforts to amplify HEV molecularly by reverse transcription and PCR have been successful and partial sequences of two HEV strains have been obtained.

Hepatitis B virus (HBV) and related hepadnaviruses. Computer and molecular biological techniques have been employed to continue our analysis of the HBV genome and its gene products. For example, the location of the X transcript promoter element was determined by computer analysis. Also, additional evidence for the evolutionary relationship between hepadnaviruses and retroviruses was obtained. Sequence heterogeneity of HBV genomes present in the serum of a patient with chronic infection was studied by molecular techniques and an unexpectedly high degree of heterogeneity was detected.

Standard methods for detection of viral DNA using the polymerase chain reaction (PCR) are time consuming and involve multiple steps during which contamination with exogenous DNA often occurs. Therefore, a simplified PCR method for detecting HBV DNA in serum was developed. The main advantages of this method are that it is relatively simple, can be performed rapidly and has a false positive rate of <0.1% in our laboratory. When sera from 84 patients were tested by this technique, HBV DNA was detected in every person who had HBsAg and hepatitis B e antigen (HBeAg) in serum and in 64% of the patients who had HBsAg, but not HBeAg, in serum. Also, 3 of 11 patients who were chronic HBV carriers and who subsequently lost HBsAg were found to have HBV DNA in serum. In contrast, each of the patients who lost HBsAg after acute HBV infection or was classified as having non-A, non-B hepatitis was negative for HBV DNA. Thus, the modified PCR technique for detection of HBV DNA-containing virions in serum is highly specific and considerably more sensitive than methods previously used for this purpose.

Woodchucks infected with woodchuck hepatitis virus (WHV) provide an experimental model for infection of humans by its close relative, HBV. The WHV genome shares approximately 65% homology with the HBV genome and the woodchuck hepatitis surface antigen (WHsAg) exhibits antigenic cross-reactivity with the HBV surface antigen (HBsAg). Also, the risk of hepatocellular carcinoma for woodchucks that are chronic carriers of WHsAg is 100%. Thus, WHV infection of woodchucks appears to be a valuable experimental system for investigating hepadnavirus replication, gene expression and development of hepatocellular carcinoma. The complete nucleotide sequence of the genomic DNA of 2 strains, WHV7 and WHV8, was determined and both were found to be 3323 nucleotides in length. Both WHV sequences were found to resemble those of other mammalian hepadnaviruses in genome organization. Both WHV DNAs were shown to be infectious when introduced directly into the liver of neonatal or adult woodchucks by transfection. Approximately one third of the transfected woodchucks became WHsAg positive, and this usually coincided with the first appearance of WHV core (WHc) antibodies. In addition, several animals became chronic WHsAg carriers. Serum from these animals was collected and pooled for use in future experiments. These pooled sera represent the equivalent of "plaque purified" virus in our model system since virus was generated from WHV DNA derived from a single transformed *E. coli*. These pooled sera should prove useful in future studies of experimental WHV infection of woodchucks.

The mutation rate of an hepadnavirus genome was estimated using virus produced by one of the transfected neonatal woodchucks that became a chronic carrier of WHV8. WHV8 DNA derived from serum virions 16 months post-transfection was cloned and the nucleotide sequence of three independent progeny genomes was compared directly with that of the input recombinant DNA. Only 3 nucleotide substitutions were detected indicating that the mutation rate of the WHV genome was $<2 \times 10^{-4}$ base substitutions/site/year. This value is slightly lower than the mutation rate previously calculated for the genomes of other animal viruses that also replicate via a polymerase that lacks proofreading functions.

One of the most pressing needs for the study of WHV has been a woodchuck hepatocyte tissue culture system for propagation and titration of this virus. Recently, hepatocytes isolated from adult woodchuck liver that had been perfused with collagenase were successfully maintained in cell culture. Preliminary studies suggest that such hepatocyte cultures support the replication of virus following transfection with HBV or WHV DNA.

Hepatitis C virus (serum non-A, non-B hepatitis virus). Hepatitis C virus (HCV) is an important human pathogen that plays a major role in post transfusion hepatitis as well as sporadic non-A, non-B hepatitis. Recently, a portion of the RNA genome of HCV was cloned molecularly and sequenced by others. A region of the predicted polyprotein sequence was found to share similarity with a non-structural protein encoded by dengue virus, a member of the flavivirus family. Computer-assisted analysis in LID indicated that HCV shares an even greater degree of protein sequence similarity with members of the pestivirus group (i.e., bovine viral diarrhea virus and hog cholera virus) that are thought to be distantly related to the flaviviruses. In addition, evidence was obtained that HCV shares significant protein sequence similarity with the polyproteins encoded by members of the picornavirus-like and alphavirus-like plant virus supergroups. These data suggest that HCV may be evolutionarily related to both plant and animal viruses.

PCR amplification was used to study the pathogenesis of HCV infection in patients and experimentally infected primates. An unexpected finding was the observation that HCV

replication could be detected as early as three days following experimental infection of chimpanzees.

Acute immune deficiency syndromes produced by lentiviruses indigenous to monkeys and cats. Three years ago a new program was initiated for the study of immune deficiency syndromes produced in monkeys and cats by lentiviruses indigenous to these species. Two experimental systems were established: (1) an immune deficiency syndrome in monkeys caused by the simian immune deficiency virus derived from sooty mangabeys, i.e., SIV_{SM}; and (2) an immune deficiency syndrome in cats caused by a feline immune deficiency virus, i.e., FIV. The syndromes produced by these viruses in their respective hosts resemble human AIDS and thus, SIV infected monkeys and FIV infected cats constitute experimental surrogates for human AIDS. Infectious molecular clones of both SIV_{SM} and FIV have been constructed and sequenced. Consistent with the view that the SIV_{SM} system is a potentially useful model was the observation that SIV_{SM} is more closely related to human immune deficiency virus type 2 (HIV-2) than any other known non-human primate lentivirus. However, SIV_{SM} was more distantly related to HIV-1 as is the case with other simian lentiviruses. Finally, FIV was observed to be even more distantly related to HIV-1, HIV-2 or the various SIVs.

Suspensions of SIV and FIV have been prepared from uncloned virus as well as virus derived from molecularly cloned cDNA. These suspensions are undergoing infectivity titration in the appropriate host. Monkeys have been successfully infected with both kinds of SIV_{SM} and some of these animals have developed AIDS. Cats have also been infected with both kinds of FIV, but to date definite disease has not been observed.

Genetic drift of SIV over a 12 month interval was documented in monkeys experimentally infected with virus derived from a molecular clone of SIV_{SM}. Significant variation was detected in the envelope glycoprotein gene but not in the integrase gene. SIV isolates from healthy infected monkeys accumulated multiple in-frame stop codons in the envelope gene, whereas isolates from immunodeficient animals did not develop such mutations.

Four biologically-active proviral molecular clones were derived directly from splenic tissue of a macaque that died from SIV infection. Each clone displayed a distinct *in vitro* tropism; two clones infected only monocyte/macrophage cells. Progeny virions from three clones have successfully infected macaques, and evaluation of these animals is ongoing. Chimeric clones are being constructed to investigate the genetic determinants of disparate tropism. In addition, 4 biologically-active molecular clones were derived from SIV/PGg, which is a variant of SIV_{SM} closely related to SIV/PBj, a rapidly lethal variant that produces an acute diarrhea that kills macaques in 8-10 days. Sequence analysis indicates that SIV/PGg contains insertions in the envelope gene and the LTR that may be responsible for the acute death phenotype. Experimental infections of macaques are planned.

An inactivated whole virus vaccine was prepared from SIV_{SM} (derived from the smH4 molecular clone). Inactivation was achieved by exposure to psoralen and UV light. To date, primary (400µg) and booster (400µg) doses adjuvanted by MDP have been given to 10 macaques. Six control animals were immunized with HBsAg (+ MDP). Preliminary results indicate that the experimental inactivated vaccine induced an appreciable level of envelope glycoprotein antibodies detectable by Western blot after the first dose. Challenge (100 MID_{50}) will be performed after a third (800µg) dose of vaccine (scheduled for 9/90).

Significant sequence diversity in the envelope glycoprotein gene was detected among FIV isolates from different geographic locations. Also, serologic evidence of infection with FIV was detected with high frequency among free-ranging, wild caught felids such as Florida panthers (*Felis concolor*).

RESPIRATORY VIRUSES

Respiratory syncytial virus (RSV): Molecular genetics and biology. A complete sequence was obtained for the 6578 nucleotide L gene (that encodes the viral polymerase) as well as the 155-nucleotide 5'-trailer region and the 44-nucleotide 3'-leader region of RSV genomic RNA (vRNA). This completes the sequence analysis of the 15,222 nucleotide RSV vRNA of strain A2 (subgroup A).

cDNAs have been constructed for the *in vitro* synthesis of synthetic vRNA-like molecules. These "vRNAs" contain a marker gene (chloramphenicol acetyltransferase) under the control of RSV transcriptive signals and flanked by the RSV 3' and 5' vRNA sequences that are thought to contain promoters that direct transcription and replication. Methods are being developed for introducing these synthetic vRNAs into RSV-infected cells so that they will associate with viral proteins and be transcribed and replicated. A cDNA of the complete RSV genomic RNA is being assembled to investigate methods for generating infectious RSV from cDNA.

Analysis of the RSV mRNAs by cDNA cloning, sequencing and related techniques had previously identified ten different mRNAs, and to date a single encoded polypeptide has been identified for each. Special attention was given to 2 of these mRNAs whose protein products were not well understood. Recently, the SH protein was shown to be an integral membrane protein that is processed by a complex intracellular pathway yielding two nonglycosylated and two glycosylated species. The processing pathway and structural differences among the different forms are conserved between the two RSV antigenic subgroups. Experiments are continuing to determine the membrane orientation, site of glycosylation, and the oligomeric status of the different forms of the SH protein. In other work, a complete nucleotide sequence was determined for the 22K (M2) gene of strain 18537, a prototype of antigenic subgroup B. Comparison with the previously-determined subgroup A (A2 strain) sequence revealed a high level (92%) of amino acid homology for M2 protein and identified a second, conserved open reading frame whose protein product remains to be identified. The high level of conservation of the M2 protein is of interest because it has been shown to be the major target for murine RSV-specific cytotoxic T cells.

RSV: Host immunity, immunoprophylaxis and vaccine-associated potentiation of pulmonary pathology. Vaccinia virus recombinants expressing the RSV F or G glycoprotein were highly immunogenic and effective in inducing resistance to RSV infection in rodents and monkeys but, surprisingly, these recombinants were not satisfactorily immunogenic or protective in chimpanzees. Although the recombinants did not induce significant protection, vaccinated chimpanzees were primed for RSV glycoprotein antigens because an extremely high booster type neutralizing antibody response occurred following RSV challenge infection. In the future it may be possible to take advantage of this priming effect.

The disparity in protective efficacy observed in cotton rats, monkeys and chimpanzees is probably due in part to differences in the capacity of vaccinia virus and RSV to replicate in these species. Vaccinia virus infection is more extensive in cotton rats, which would favor

greater immunogenicity of the vaccinia recombinants in this species. Although, the replication of vaccinia virus recombinants appeared to be similar in monkeys and chimpanzees, the immunogenicity of the RSV glycoproteins expressed by the recombinants was considerably less in the latter species for reasons that remain unclear. Nonetheless, this difference in immunogenicity of RSV glycoproteins in two primate species may have implications for the future usefulness of vaccinia virus-RSV recombinants in humans. Finally, it should be noted that RSV grows to higher titer in chimpanzees than in rodents or monkeys, thereby placing greater demands upon a vaccine as regards protective efficacy in chimpanzees.

In studies designed to investigate the participation of individual RSV proteins in host immunity, recombinant vaccinia viruses were constructed that individually expressed seven additional RSV genes, namely the 1C, 1B, N, P, M, M2, and SH genes. Construction of a recombinant for the remaining gene, L, is underway. These recombinants are being evaluated for immunogenicity, protective efficacy, and possible disease potentiation in rodents. In recently completed studies, the SH and N proteins did not induce significant protective immunity, in contrast to the high levels of protective immunity elicited by the F and G glycoproteins. In other studies the M2 protein was found to be the major viral target for RSV-specific murine cytotoxic T lymphocytes, with F and N being secondary targets, but the importance of cytotoxic T cells in immunity or disease enhancement remains to be defined.

RSV: Immunotherapy. The efficiency of topical immunotherapy for RSV infection was significantly increased by two modifications of previous methodology. First, a mixture of RSV F monoclonal antibodies directed at the major conserved neutralization epitopes on this glycoprotein was highly effective in topical immunotherapy of RSV infection in the cotton rat. Second, delivery of RSV antibodies directly into the lungs in a small particle aerosol (<2 mu) was also effective therapeutically. The use of monoclonal antibodies should decrease the amount of IgG required for therapy by 2 orders of magnitude. In other studies in cotton rats, parainfluenza virus type 3 (PIV3) antibodies were also shown to be therapeutic when administered directly into the respiratory tract. Thus, the usefulness of topical immunotherapy is not limited to RSV. It is likely that this approach will prove to be effective for other respiratory viral pathogens whose pathogenic effects are also limited to the cells that line the lumen of the lower respiratory tract.

RSV: Enhancement of pulmonary pathology by prior immunization with inactivated virus or virus subunits. Enhanced pulmonary pathology was observed in cotton rats immunized with formalin-inactivated RSV or purified RSV fusion (F) protein and challenged intranasally with RSV 3 or 6 months later. This constitutes the first evidence that immunization with the F glycoprotein alone can induce a potentiated pathological response to subsequent RSV infection. This study also showed that potentiation is not unique to formalin-treated antigen, but instead might be a more general problem associated with RSV subunit vaccines. Enhanced pulmonary pathology was not observed previously when animals immunized with purified RSV F were challenged with RSV one month later. This observation indicates the importance of the temporal relationship between immunization and subsequent virus infection in the development of enhanced pulmonary pathology. A similar situation was observed almost 25 years ago with the licensed inactivated measles virus vaccine and thus, caution should be exercised in the evaluation of a purified RSV F glycoprotein vaccine in humans. Currently, the ability of adenovirus-RSV recombinants and vaccinia virus-RSV recombinants to potentiate pulmonary pathology is under investigation. These studies represent an essential phase of the orderly evaluation of candidate RSV vaccines prior to initiation of clinical trials.

Passively acquired RSV F antibodies can suppress the neutralizing antibody response of cotton rats to purified RSV F glycoprotein. With lower doses of F (0.2 μg to 1.7 μg) there was a 7 to 8-fold suppression, while with high doses of F (5 to 15 μg) suppression was significantly less, i.e., 2 fold.

Parainfluenza virus type 3 (PIV3): Molecular biology. Human parainfluenza virus type 3 (PIV3), a paramyxovirus, is second in importance only to respiratory syncytial virus (RSV) as a major etiologic agent of pediatric viral respiratory tract disease. The intracellular synthesis and maturation of the hemagglutinin-neuraminidase (HN) protein of PIV3 was investigated with regard to the kinetics of folding and the formation of homooligomers that constitute the mature HN virion spike. Folding of the HN protein, as monitored by the acquisition of immunoreactivity with conformation-dependent antibodies and by electrophoretic mobility during nonreducing SDS-PAGE, was found to have a half-time of approximately 15 min. Post-infection cotton rat PIV3 antisera and HN-specific murine monoclonal antibodies (mabs) from a previously-described panel showed little or no reactivity with the newly-synthesized, unfolded protein, illustrating the conformational nature of the major HN epitopes. Chemical cross-linking and sucrose sedimentation analysis of the HN protein provided evidence suggesting that the protein forms homotetramers. In previous studies, other viral glycoproteins analyzed following short pulse labeling were shown to exist primarily as unfolded monomers which oligomerized only after the monomers had folded into an essentially mature conformation. In contrast, newly-synthesized unfolded intracellular PIV3 HN protein was found exclusively in the form of oligomers that were indistinguishable in sedimentation profile and stability from those of the mature protein.

Neutralizing monoclonal antibodies specific for the fusion (F) glycoprotein of human parainfluenza type 3 virus (PIV3) were used to select neutralization-resistant antigenic mutants. Sequence analysis of the F gene of the mutants indicated that resistance to antibody binding, antibody-mediated neutralization or to both was a result of single specific amino acid substitutions within neutralization epitopes of the F1 and F2 cleavage subunits of the fusion protein. Comparison of the location of PIV3 F neutralization epitopes to the corresponding sites on the F protein of Newcastle disease virus and Sendai virus indicated that the antigenic organization of the fusion proteins of these three paramyxoviruses was similar. Futhermore, some of the PIV3 F epitopes recognized by syncytium-inhibiting monoclonal antibodies were located in an F1 cysteine cluster region that corresponds to an area of the measles virus F protein involved in fusion activity.

PIV3: Human immune response. More than 90% of infants or young children who underwent primary infection with PIV3 developed antibodies to 4 of the 6 hemagglutinin-neuraminidase (HN) antigenic sites (including 3 of the 4 neutralization sites), whereas the antibody response to F antigenic sites was less extensive and varied considerably from person to person. Also, the response to antigenic sites of both F and HN was suppressed in infants who possessed maternally-derived serum PIV3 antibodies. The restriction of immune response to the F glycoprotein during primary infection and the immunosuppressive effect of maternally-derived PIV3 antibodies present in the serum of young infants may play a role in the susceptibility of infants and young children to reinfection with PIV3.

PIV3: Cold-adapted PIV3 mutant. A cold-adapted mutant of human PIV3 selected after 12 passages at suboptimal temperature (cp12) was shown to be attenuated, immunogenic, and protective in chimpanzees. These observations form the basis for evaluation of this mutant and

its more attenuated progeny (cp18 and cp45) in humans, including seronegative infants and children.

Attenuated influenza A reassortant virus vaccine strains derived from the cold-adapted (*ca*) human influenza A virus donor or the avian influenza A virus donor. Live attenuated bivalent *ca* influenza A reassortant virus vaccine was shown to be less immunogenic in the elderly than inactivated influenza A virus vaccine. Little increase in immunogenicity was observed when parenterally administered inactivated vaccine was used in combination with live, bivalent *ca* influenza A virus vaccine administered intranasally. During the course of these studies it was observed that the elderly possess non-hemagglutination-inhibiting antibodies that correlate with resistance to infection by live attenuated vaccine virus. These observations indicate that the *ca* influenza A reassortant virus vaccines will find their greatest usefulness in infants, children and young adults in whom these vaccines are satisfactorily immunogenic.

Previously, *ca* human influenza A virus reassortants and avian-human (*ah*) influenza A virus reassortants bearing H3N2 surface glycoproteins were shown to be safe, satisfactorily attenuated and immunogenic when tested in seronegative infants and young children. These reassortants, that contained the six internal protein genes of the attenuated donor virus and the 2 surface glycoprotein genes of the wild type human influenza A/Bethesda/85 H3N2 virus, could not be distinguished as regards attenuation and immunogenicity in susceptible infants and children. However, this was not the case when similar reassortants derived from a wild type human influenza A H1N1 virus were evaluated in seronegative infants and children. Both reassortants were immunogenic but the *ah* influenza A H1N1 reassortant virus induced a transient febrile response in 24% of vaccinees, whereas the *ca* influenza A H1N1 reassortant virus was satisfactorily attenuated. It would appear that an interaction of the hemagglutinin and neuraminidase surface glycoproteins of the human wild type H1N1 virus with the six internal viral proteins of the avian influenza A virus donor strain adversely influenced the reactogenicity of the reassortant derived from these two viruses. Because the avian influenza A virus donor does not consistently confer satisfactory attenuation on influenza A viruses of varying subtype, it is not a suitable donor virus for use in constructing live influenza virus vaccines. In contrast, the influenza A/Ann Arbor/6/60 *ca* virus remains an acceptable donor since it consistently confers attenuation on reassortants derived from a variety of wild-type human influenza A H1N1 and H3N2 viruses.

Studies were initiated to investigate the genetic stability of the genes of the cold-adapted (*ca*) influenza A/Ann Arbor/60 donor virus that confer attenuation on reassortant viruses. Initially, a single gene reassortant was prepared that contained the PB2 gene of the *ca* donor virus in a background of 7 other genes derived from a human wild type influenza A virus. The PB2 protein is a constituent of the viral polymerase complex that also includes the PB1 and PA proteins. The PB2 gene derived from the *ca* donor virus specifies a temperature sensitivity (*ts*) phenotype. This allowed us to identify revertants by their *ts*+ phenotype. Following selection of 4 independent *ts*+ revertants genetic analysis indicated that each revertant had developed a suppressor ("second site") mutation in the PA gene. In contrast, sequence analysis of each revertant indicated that the sequence of the mutant PB2 gene was unaltered. These observations suggested that a suppressor mutation developed in another protein of the polymerase complex (i.e., PA) of the 4 revertants and this mutation corrected the defect in polymerase activity specified by the mutant PB2 gene.

Although the avian influenza A/Mallard/78 virus did not prove to be an acceptable donor for use in preparing live vaccine strains, its attenuation phenotype appeared to be stable. The

M or NP gene of the avian influenza A/Mallard/78 donor virus was transferred into a reassortant virus in which the other 7 genes were derived from a wild type human influenza H3N2 virus. The M and NP genes, which are the major attenuating genes of the avian influenza A donor virus, were stable genetically after 5 serial passages of these reassortant viruses in monkeys. Furthermore, the reassortant viruses retained their attenuation phenotype following the 5 serial passages in monkeys. Another interesting property of one of the genes of this donor virus was discovered during the past year. A single gene reassortant, that contained the PB2 gene of the attenuated avian influenza A/Mallard/78 donor virus and 7 other genes derived from the virulent human influenza A/LA/87 (H3N2) virus, was shown to be markedly restricted in its replication in mammalian cells, but this reassortant replicated efficiently in avian cells. Studies are in progress to determine if this gene constellation will consistently attenuate wild type human influenza A viruses for monkeys and humans through a similar host range effect.

DENGUE VIRUSES

Considerable progress was made during FY 1990 in elucidating the strategies employed by dengue virus for expression and processing of its 10 viral proteins. In addition, one of our major research objectives was achieved, namely the construction of full-length cloned dengue DNA which can serve as a template to produce full-length RNA transcripts that are infectious for permissive cells in culture. As a consequence we are now able to introduce specific mutations into cloned dengue DNA and transfer these mutations into infectious virus by transfection of full-length RNA transcripts. This will allow us to probe the dengue viral genome and produce specific mutations throughout the genome that can be evaluated for their usefulness in the development of a safe, effective live virus vaccine.

Expression and processing of viral proteins. The dengue virus positive strand RNA genome codes for a single long polyprotein with the gene order NH_2-C-PreM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5. Expression of viral proteins takes place by specific proteolytic processing of the polyprotein. Research performed in LID last year demonstrated that three distinct strategies are employed for post-translational cleavage of the viral polyprotein. The first type of cleavage occurs after a long hydrophobic sequence; this strategy is used to generate structural proteins PreM and E as well as cleavage of the junction between non-structural proteins NS4A and NS4B. This form of cleavage appears to occur co-translationally and is catalyzed by host cell signalase. The second type of cleavage takes place after a di-basic amino acid sequence such as Lys-Arg or Arg-Arg and it appears to be responsible for the processing of most non-structural proteins. In addition, a third type of cleavage occurs at the NS1-NS2A junction because this region does not have a hydrophobic domain or a di-basic amino acid motif.

A more detailed understanding of the cleavage between the first 2 non-structural proteins, NS1 and NS2A, was achieved by deletion analysis in which mutant sequences were expressed by a vaccinia virus recombinant. First, it was shown that cleavage is effected by a *cis-acting* function provided by the 70% N-terminal region of NS2A. Second, the C-terminal 8 amino acids of NS1 that precede the NS1-NS2A junction were found to be required for cleavage. Comparison of this 8 amino acid sequence of dengue type 4 virus and analogous sequences of other flaviviruses indicated that there is a consensus sequence in which amino acid positions -7, -5, -3 and -1 with respect to the cleavage site are completely conserved. The importance of this amino acid motif for optimal cleavage was demonstrated during a study in which amino acid substitutions at positions -3 or -1 abrogated cleavage. In contrast, amino acid substitutions at position -2, a position that is not conserved among flaviviruses, had only a slight to moderate

effect on cleavage. Although position +1 (i.e., immediately downstream of the cleavage site) is not conserved, substitution at this position reduced cleavage 42 to 90%.

NS3 exhibits limited sequence homology to serine proteases prompting others to propose that NS3 is a protease. Support for view was provided by studies in LID in which it was shown that the N-terminal 30% of NS3 acts *in trans* to cleave the NS2A-NS2B, NS2B-NS3, NS3-NS4A and NS4B-NS5 junctions. Cleavage of these junctions is somewhat more complex, however, because NS2B, also acting *in trans*, appears to be required.

Immunogenicity and efficacy of protective antigens. Although the envelope (E) glycoprotein expressed by a vaccinia virus or baculovirus recombinant induced significant resistance in mice to lethal dengue virus challenge, this protective antigen induced little if any E-specific antibodies. Attempts were made to prime an E antibody response by various sequential patterns of immunization with vaccinia virus-E, baculovirus-E or live dengue virus administered parenterally. None of these sequential immunizations primed a response to E.

Next, the antigenic structure of dengue envelope (E) glycoprotein was investigated with the goal of increasing its immunogenicity and protective efficacy. Recombinant vaccinia viruses were constructed that contained dengue cDNA coding for C-terminally truncated E that ranged in length from 9% to 99% of the complete sequence. With the exception of 59%E, only E constructs greater than 79% were able to bind hyperimmune polyvalent dengue antibodies. Arg present at amino acid position 392 immediately downstream of the 79%E C-terminus, appeared to be critical for proper conformation and antigenic structure required for optimal binding of these antibodies. Studies in mice indicated that only truncated E's that were recognized efficiently by polyvalent antibodies induced a high level of resistance to dengue virus encephalitis.

Full-length E was retained intracellularly, and only truncated E ranging from 59% to 81% in length was secreted extracellularly. Serendipitously, it was observed that 79% E-RKG, that was constructed from 79% E by the addition of the next 3 authentic downstream amino acids, had acquired the unique property of being expressed in high concentration on the surface of recombinant virus-infected cells, presumably being inserted into the plasma membrane by a C-terminal membrane anchor. 81% E was also expressed at the cell surface, but only in low concentration. When the various deletion mutants were studied in mice only 79% R-RKG was able to induce an appreciable E antibody response suggesting that cell surface expression was responsible for its enhanced immunogenicity. Finally, passive immunization studies in mice involving serum transfer indicated that serum antibodies to E played a major role in the complete or nearly complete resistance to dengue virus challenge induced by certain vaccinia virus-truncated E recombinants. Significantly, mice immunized with vaccinia recombinant v(79%E-RKG), or with v(81%E), showed few, if any, signs of encephalitis (ruffled fur, hunched back) after dengue virus challenge. In contrast, other groups of mice immunized with recombinant v(88%E), v(94%E), or v(100%E) developed signs of encephalitis but survived challenge. Thus, in addition to being more immunogenic than longer E constructs, 79%E-RKG and 81%E induced a higher level of resistance to experimental dengue virus disease that provided protection against signs of disease as well as death.

The pre-membrane (pre-M) structural glycoprotein of dengue virus is cleaved during virus maturation to yield a membrane (M) protein that becomes part of the virus, leaving a residual glycoprotein segment (Mresid) that is not associated with virus. Following immunization with a vaccinia virus recombinant expressing PreM, M or M resid, almost all mice previously

inoculated with vaccinia virus-dengue type 4 virus recombinant vD4 preM or vD4 M survived subsequent intracerebral homologous virus challenge, while control mice or mice previously inoculated with vD4 Mresid were not protected. Serum transfer experiments provided evidence that protection was mediated by antibodies. The role of pre-M as a protective antigen was investigated further by constructing vaccinia recombinants expressing dengue type 2 virus (D2) structural proteins. A recombinant that expressed both D2 pre-M and E glycoproteins induced solid resistance to homologous virus challenge, but recombinants that expressed E or pre-M alone were only partially protective. Protection was enhanced when mice were immunized with both vD2 pM and vD2 E recombinants. Thus, M constitutes a third, previously unrecognized, protective antigen of dengue virus that must be taken into consideration during the development of dengue virus vaccines.

Full-length RNA transcripts of cloned dengue DNA that are infectious for cell culture. One of our major research objectives for the dengue viruses has been the construction of full-length cloned dengue DNA that could be used for the transcription of full-length RNA transcripts able to initiate dengue virus infection following transfection of permissive cells in culture. Our strategy was to use the pBR322 plasmid vector to clone full-length dengue DNA from two fragments, each containing approximately one-half of the dengue genomic sequence. The full-length dengue virus cDNA was placed under the control of an SP6 polymerase promoter and a unique Asp 718 cleavage sequence was inserted at the 3'-end of the dengue sequence for use in producing run-off transcripts. Asp 718 linearized DNA was then used as a template for *in vitro* transcription of RNA using SP6 polymerase. m^7G capped GTP was added to the transcription reaction to produce full-length dengue RNA transcripts containing a cap structure at the 5' end. Transfection of simian LLCMK2 cells with the transcription products yielded infectious dengue virus as indicated by the appearance of dengue virus antigens in a majority of cells. Progeny dengue virus was recovered at a titer of 10^5pfu/ml from a lysate of transfected cells. Treatment of the transcription products with DNase did not affect infectivity, while RNase treatment completely abolished infectivity. Formal proof for the infectivity of the RNA transcripts was provided by engineering two silent mutations into the dengue cDNA and demonstrating the presence of these mutations in dengue virus recovered from cells transfected with the mutant RNA transcripts. Experiments are underway using the full-length dengue cDNA to engineer mutations at strategic sites in the dengue viral genome. Subsequently, infectious dengue virus containing the mutations introduced into dengue cDNA will be evaluated for various biological and immunological properties *in vitro* as well as alteration in virulence for experimental animals. This strategy may yield attenuated mutants that are satisfactory for use in a safe, effective, live virus vaccine.

Cross reactive antibodies to clotting factors present in serum of dengue virus-infected individuals. The major antigen of the virus, against which neutralizing, hemagglutination-inhibiting, and flavivirus cross-reactive antibodies are directed, is the envelope glycoprotein (E). Computer analysis revealed a 20 residue region of similarity in amino acid sequence between the dengue type 4 E and a family of clotting factors, including plasminogen, the prime mediator of fibrinolysis. Using synthetic peptides in ELISA, E antibodies that potentially bind plasminogen were detected in 75% of 40 Thai patients acutely infected with dengue virus type 1, 2, 3, or 4. Cross-reactivity of dengue antibodies with plasminogen was shown to be specific for the related sites in E and plasminogen.

ROTAVIRUSES

Live rotavirus vaccines. Rotaviruses are the single most important etiologic agents of severe diarrheal illness of infants and young children worldwide. Thus, there is an urgent need for a rotavirus vaccine that can prevent severe rotavirus diarrhea during the first two years of life when this illness is most serious. The "Jennerian" approach to vaccination, which involves use of a live non-human rotavirus, is currently being evaluated in clinical trials of a rhesus rotavirus (RRV) strain. This strategy has had limited success because serotype-specific immunity against all 4 epidemiologically important human rotavirus serotypes (specified by outer capsid protein VP7) could not be achieved consistently in infants less than 6 months of age, most of whom had not undergone prior rotavirus infection. Although, highly effective protection was observed when the simian RRV vaccine (VP7 serotype 3) was used to immunize young infants who were later exposed to human rotavirus strains of the same VP7 serotype, in other trials in which VP7 serotype 1 rotaviruses predominated in the community, vaccine efficacy was variable. As a consequence, most recent clinical trials have evaluated a "modified Jennerian" approach in which a quadrivalent vaccine of broader antigenic coverage (that includes viruses of VP7 serotype 1, 2, 3, and 4) was used for immunization. This vaccine contains RRV (serotype 3) and reassortant rotaviruses containing 10 RRV genes and a single human rotavirus gene that encodes a VP7 with serotype 1, serotype 2, or serotype 4 specificity. Phase 1 studies of individual reassortants representing VP7 serotype 1 (DxRRV), 2 (DS-1xRRV), or 4 (ST3xRRV) indicated that each resembled the RRV vaccine with respect to reactogenicity and antigenicity. Therefore, the three reassortants plus RRV (serotype 3) were combined into a single quadrivalent vaccine and the mixture was evaluated in phase 1 studies for reactogenicity and antigenicity. The quadrivalent vaccine was observed to be similar to RRV with regard to attenuation. Initially, seroresponse to the individual components of the quadrivalent vaccine (10^4 pfu for each component) was disappointing. However, the use of a two dose (10^5 pfu per dose) schedule increased immunogenicity so that 50% or more of the vaccinees developed neutralizing antibodies to each of the 4 serotypes included in the vaccine. Preliminary analysis of the protective efficacy of these vaccines indicated that in Rochester the DxRRV (VP7 serotype 1) reassortant (as well as RRV [VP7 serotype 3]) induced protection against rotavirus diarrhea associated predominantly with VP7 serotype 1 strains. In Finland the DxRRV or DS-1xRRV (VP7 serotype 2) reassortant vaccine also induced protection against rotavirus diarrhea that was associated predominantly with VP7 serotype 1 strains.

In addition, clinical trials are underway to evaluate a naturally attenuated human neonatal rotavirus strain, M37, that has a serotype 1 VP7 and a unique VP4 that is shared by other neonatal attenuated strains. Phase 1 trials of the M37 human rotavirus live vaccine have been completed successfully. The M37 rotavirus vaccine was shown to be safe and immunogenic in a clinical study involving 150 young infants (2-4 months of age) and as a consequence phase 2 efficacy trials are now in progress.

Antigenic polymorphism of the rotavirus outer capsid protein VP4. The outer capsid protein VP4 is one of two major independent protective antigens of rotavirus, the other being the outer capsid protein VP7. Six distinct VP7 serotypes have been identified among the human rotaviruses but only four of these serotypes (1, 2, 3, and 4) appear to be responsible for the vast majority of rotavirus illnesses. In contrast, the extent of antigenic diversity and the distribution of VP4 serotypes among human rotaviruses have not been defined. Because VP4 is an important rotavirus protective antigen, studies were initiated to address this deficiency.

A cDNA clone representing the VP4 gene of symptomatic human rotavirus strain KU (VP7 serotype 1) or DS-1 (VP7 serotype 2) or asymptomatic human rotavirus strain 1076 (VP7 serotype 2) was constructed and inserted into a baculovirus expression vector under the control of the polyhedrin promoter. The resulting recombinants expressed the appropriate authentic VP4 protein. Guinea pigs immunized with these VP4 proteins developed specific antibodies which neutralized infectivity of the homologous rotavirus. These antisera were then used in neutralization tests to define the extent and distribution of VP4 antigenic polymorphism among human rotaviruses. Three distinct serotypes of the VP4 outer capsid protein were identified among 17 human rotavirus strains that had previously been assigned to 5 distinct VP7 serotypes. In addition, one of the VP4 serotypes contained two subtypes. For the most part, VP4 serotype segregated independently of VP7 serotype. Among the 10 rotavirus strains whose VP4 gene had been sequenced previously, there was complete concordance between VP4 serotype as determined by neutralization and previous classification of strains into genetic groups based on VP4 amino acid homology. Thus, rotaviruses that exhibited a VP4 amino acid homology of 89% or greater belonged to the same VP4 serotype as determined by neutralization.

During rotavirus maturation two subunits of VP4, i.e., VP8 and VP5, are generated by proteolytic processing. The VP5 and VP8 cleavage subunits were separately expressed in *E. coli* as part of an initial effort to define the antigenic domains of VP4. Immunization of guinea pigs with the VP5 or VP8 of strain KU, DS-2 or 1076 induced antibodies that neutralized the rotavirus from which the VP4 subunits were derived. Cross-immunoprecipitation and reciprocal neutralization assays using antisera to the VP5 and VP8 expressed proteins formally demonstrated that the VP8 subunit contains the major antigenic site(s) responsible for serotype-specific neutralization of rotavirus mediated by VP4, whereas the VP5 subunit is responsible for the cross-reactivity observed among strains that belong to different VP4 serotypes. In addition, it was shown that the serotype-specific VP8 subunit was considerably more immunogenic than the cross-reactive VP5 subunit.

In other studies the binding of the VP4 protein to susceptible MA104 cells was demonstrated using VP4 expressed by a baculovirus recombinant. Moreover, binding assays using the VP5 or VP8 subunit of VP4 synthesized separately *in vitro* indicated that the VP5 subunit alone was responsible for the adsorption of the VP4 outer capsid protein to susceptible host cells.

Immunological responses to epitopes on VP7 and VP4 following infection or vaccination. Several monoclonal antibodies have been adapted for use in an epitope-blocking immunoassay in order to analyze antigenic site-specific antibody responses to infection with wild type rotaviruses or attenuated rotavirus vaccine strains. This technique is being utilized to analyze sera from rotavirus vaccine trials so that the immune responses important in vaccine induced protection can be identified. Using this assay it was possible to partially characterize the repertoire of serum antibody responses of infants or adults following rotavirus immunization. Administration of a single dose of the attenuated rhesus rotavirus (RRV) or a human-RRV reassortant rotavirus induced vaccine serotype-specific antibody responses in infant vaccinees less than six months old. However, heterotypic responses were not induced. Thus, infant vaccinees without prior rotavirus experience mount an immune response that may not be sufficiently cross-reactive to protect against heterotypic rotaviruses. This was confirmed by the analysis of sera from "rotavirus vaccine failures". After vaccination these infants failed to develop an antibody response for the serotype of the naturally circulating heterotypic rotavirus that subsequently caused illness.

Sera from adult volunteers who were challenged previously with a virulent human VP7 serotype 1 rotavirus were also analyzed by the epitope blocking assay. Resistance to illness was associated with the presence of blocking antibody to a previously defined epitope on VP7 prior to challenge. Sera from various vaccine trials will be analyzed to determine whether antibody specific for this epitope is an accurate predictor of vaccine efficacy.

Genetic determinants of virulence. Previously, it was shown that the VP4 and VP7 genes play an important independent role in: (i) the growth of rotavirus in the gut of gnotobiotic newborn piglets, and (ii) the induction of diarrhea in these animals. This was demonstrated in studies of reassortants derived from human rotavirus DS-1 (that does not cause diarrhea in piglets) and porcine rotavirus SB1A (that does cause diarrhea in piglets). Thus, human DS-1-porcine SB1A reassortants lost virulence when the DS-1 VP4 was substituted for the SB1A VP4 or when the DS-1 VP7 was substituted for the SB1A VP7. This year, we observed that piglets fed reassortant 41-27, which contained gene number ten of DS-1 and the remaining genes from SB1A, did not show any clinical signs or shed virus. Thus, it appears that gene ten, in addition to the VP4 and VP7 genes, plays an important role in: (i) the growth of rotavirus in the gut and (ii) the induction of diarrhea.

Non-group A rotaviruses. Although the major goal of the Epidemiology Section is the development of a vaccine against group A rotavirus, it is essential to develop reagents for the study of other groups of rotaviruses (B-F) since viruses belonging to 2 of these groups, B and C, have also been implicated as etiologic agents of diarrheal disease in humans in Asia. It is also possible that non-group A rotaviruses will emerge as important world-wide pathogens after vaccines for group A rotaviruses are used extensively. Using an improved strategy for cloning rotavirus genomic RNA of unknown sequence, cDNA libraries of a porcine group C strain and a human group C rotavirus isolate were then generated and several partial clones specific for different genomic segments were selected and sequenced. Using this sequence information coupled with the polymerase chain reaction (PCR) amplification technique, it was possible to generate complete clones of the 8th gene of both human and porcine group C rotaviruses and obtain evidence this group C gene encodes the VP7 outer capsid protein, a major protective antigen. In addition, the porcine and human group C rotaviruses were found to be closely related genetically and quite distinct from group A rotaviruses. It appears that the porcine and human group C rotaviruses evolved from a common ancestral source.

HONORS AND AWARDS

Robert M. Chanock

Co-organizer, Cold Spring Harbor Conference on Modern Approaches to Vaccines Including Prevention of AIDS, September 1989.

Member of the National Academy of Sciences, Committee on International Security and Arms Control (CISAC), Working Group on Biological Weapons Control, 1989-90.

Participated in Meeting of Working Groups of the US National Academy of Sciences and the Academy of Sciences of the USSR on Biological Weapons Control, Moscow and Leningrad, October 6-7, 1989.

Participated in site visit to Institute of Military Medicine in Leningrad, USSR, October 8, 1989.

Invited to summarize, NIAID-BOB, FDA Workshop on Hepatitis A Virus Infection and Control, Washington, DC, November 12, 1989.

Member of the Board of Scientific Governors, Research Institute Scripps Clinic, January 11, 1990.

Co-organizer of Sixth Annual Symposium on Animal Virology, Wallace Rowe Symposium, Lister Hill Auditorium, NIH, February 5-6, 1990.

Invited speaker, Talamo Lecturer of the American Thoracic Society, "The development of vaccines to prevent respiratory viral diseases," May 20-24, 1990, Boston, MA.

Invited guest, 50th year anniversary of Armed Forces Epidemiological Board, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, June 28, 1990.

Invited to organize and chair the workshop "New trends in vaccine development", VIIIth International Congress of Virology, Berlin, West Germany, August 26-31, 1990.

Albert Z. Kapikian

Member, US Panel of Joint Viral Diseases Panel of US-Japan Cooperative Medical Sciences Program.

Co-chairman of session on Viral Gastroenteritis at Annual Meeting of Joint Viral Diseases Panels of US-Japan Cooperative Medical Science Program, Annapolis, MD, August 20-23, 1989. Chairman of the session on August 22, 1989.

Invited to present Pediatric Grand Rounds on Enteric Diseases in Children at the Uniformed Services University of the Health Sciences, Bethesda, MD, November 9, 1989.

Invited to consult on United Medical Laboratories regarding Rotavirus laboratory practices by Wyeth-Ayerst Research, McLean, VA, December 15, 1989.

Invited to participate in workshop on "The Electronmicroscopic Detection of Viral Agents of Gastroenteritis" at the Centers for Disease Control, Atlanta, GA, January 30-February 1, 1990.

Brian R. Murphy

Invited speaker, VI International Conference on Comparative and Applied Virology, Baniff, Alberta, Canada, October 15-21, 1989. Plenary Session 2, "The factors leading to the generation of novel influenza A viruses in humans."

Invited to lecture, Department of Microbiology and Immunology, University of Maryland, School of Medicine, October 30, 1989.

Invited to review the Discovery Program of the Biotechnology and Microbiology Division of Wyeth-Ayerst Research, November 9-10, 1989.

Invited Visiting Research Professor, Children's Hospital National Medical Center, April 2, 1990. "Evaluation of live attenuated influenza A virus vaccine in infants and children".

Co-chairman, "Development of new vaccines against measles", World Health Organization, Geneva, May 26-27, 1990.

Chairman for Steering Committee on Acute Respiratory Diseases and Measles, Geneva, May 28-29, 1990.

Invited to participate in the 7th session of the Scientific Advisory Group of Experts (SAGE) of the Programme for Vaccine Development and Transdisease Vaccinology, World Health Organization, Geneva, June 25-27, 1990.

Invited to participate in pre-SAGE round table discussions on "Innovative approaches for the development of third-world vaccines", Institut Henry-Dunant, Geneva, June 24, 1990.

Invited presenter, VIIIth International Congress of Virology, Berlin, West Germany, August 26-31, 1990. "The attenuation of wild-type influenza A virus by genetic reassortment with attenuated influenza A donor viruses."

Robert H. Purcell

Invited speaker, 3rd Annual Scientific Meeting of the Hong Kong Association for the Study of Liver Diseases, Hong Kong, December 7-9, 1989.

Invited speaker, 1990 UCLA colloquium on Animal Models of Human Viral Diseases, March 31-April 6, 1990, Keystone, CO. "Primate models for viral hepatitis".

Invited Committee member, 1990 International Symposium on Viral Hepatitis and Liver Disease, Houston, TX, April 5-8, 1990.

Invited to present, Conference on Controversies in Infectious Diseases, Palm Springs, CA, May 3-5, 1990. "Hepatitis viruses: potential for control".

Organizer and Chairman, Workshop on HBV and HDV, VIIIth International Congress of Virology, Berlin, West Germany, August 26-31, 1990.

Suzanne U. Emerson

Invited speaker, Seminar in Indo-US Vaccine Programme Workshop, "New development in vaccinology", New Delhi, India, October 25, 1989. "Genes of hepatitis A involved in virulence and adaptation to cell culture."

Invited speaker, NIAID-BOB, FDA workshop on Hepatitus A Virus Infection and Control, Washington, DC, November 12, 1989. "Genes of hepatitis A involved in virulence and adaptation to cell culture." Chaired session of "Identification of shed virus."

Invited speaker, Department Seminar at the University of South Carolina School of Medicine, March 26, 1990. "Hepatitis A virus: molecular studies relevant to vaccine development."

Invited speaker, Seminar at the International Symposium on Viral Hepatitis and Liver Disease, Houston, TX, April 5, 1990. "Effect of 5' mutations on the host range of hepatitis A virus in cell culture."

Roger H. Miller

Invited speaker, Lederle Laboratories, Pearl River, NY, June 1990.

Invited speaker, Conference on Hepacellular Carcinoma in North America, Bethesda, MD, September 1990.

Invited speaker, National Institutes of Health Research Day, Bethesda, MD, September 1990.

Invited contributor to the Tenth Anniversary edition of *Seminars in Liver Disease*.

Ching-Juh Lai

Invited speaker, Session on arboviruses at the Annual Meeting of Joint Viral Diseases Panels of U.S.-Japan Cooperative Medical Science Program, Annapolis, MD, August 20-23, 1989.

Invited speaker, Cold Spring Harbor Conference on Modern Approaches to Vaccines including Prevention of AIDS, September, 1989.

Invited speaker, 1990 Annual Meeting of the American Society for Virology. Salt Lake City, Utah, July 8-12, 1990.

Awarded, a three-year research contract for "Development of Safe and Effective Vaccines for Dengue Virus Diseases by Recombinant Baculovirus", by the U.S. Army Medical Research and Development Command.

Awarded, a research grant for "Mapping protective epitopes of dengue virus envelope protein and non-structural protein NS1", by the World Health Organization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00308-09 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

In Vitro and *In Vivo* Studies of Hepatitis A Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID
Others:	Stephen M. Feinstone, M.D.	Medical Officer	LID, NIAID

COOPERATING UNITS (if any)

Smith Kline RIT (d'Hondt); FDA (Daemer); Fairfield Hospital, Melbourne, Australia (Gust); AFIP (Ishak); WRAIR (Sjogren)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

INACTIVE 1989-90

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00309-09 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hepatitis A Virus (HAV)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Stephen M. Feinstone, M.D.	Medical Officer	LID, NIAID
Others:	Suzanne U. Emerson, Ph.D.	Microbiologist	LID, NIAID
	Czeslaw Wychowski, Ph.D.	Visiting Associate	LID, NIAID
	Edward Cox	Guest Researcher	LID, NIAID
	Patricia Shields	Guest Researcher	LID, NIAID
	Robert Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS *(if any)*

Johns Hopkins Medical School (Karron); Harvard Medical School (Cohen); LIG, NIAID (Maloy); Research Institute of Scripps Clinic, La Jolla, CA (Lerner); University of North Carolina (Lemon)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED 1989-90

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00311-09 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Search For New Hepatitis Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS (if any)

National Institute of Virology, Pune, India (Arankalle); USUHS, Bethesda, MD (Legters); WRAIR, Washington, DC (Ticehurst, Sjogren); Hospital for Sick Children, Toronto, Canada (Phillips); CDC (Bradley); Gene Labs, Inc. (Reyes)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	0.3	1.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In 1980 we described a new form of viral hepatitis caused by a previously unrecognized hepatitis virus with epidemiologic characteristics of HAV (i.e., the virus is fecal-orally transmitted, produces water-borne epidemics and is probably responsible for a significant proportion of endemic hepatitis) but not related to HAV. The disease has been called epidemic or enterically transmitted non-A, non-B hepatitis or hepatitis E. Such hepatitis is both epidemic and endemic throughout the Indian subcontinent, central Asia, parts of the Middle East and northern Africa, with probable extension to West Africa. Epidemics of the disease have also occurred in Mexico. The putative etiologic agent, a 27-30nm nonenveloped virus, was identified in a volunteer study in 1983 and similar virus-like particles have been detected in feces of cases in a number of other outbreaks. The small quantity of such virus-like particles available for study has limited progress but it is now possible to biologically amplify the virus by collecting bile from experimentally infected cynomologous monkeys. These monkeys as well as chimpanzees develop an elevation of liver enzymes 28 days past inoculation. Efforts to amplify HEV molecularly by reverse transcription and PCR have been successful and partial sequences of two HEV strains have been obtained.

In 1989 a possible paramyxovirus etiology for neonatal (giant cell) hepatitis was proposed, based on electron micrographic changes seen in the livers of patients with this disease. Collaborative studies, including attempts to transmit the disease to primates, have been initiated.

The objectives of this project are to identify and characterize new etiologic agents of hepatitis and to develop useful assays for diagnosis of infection and seroepidemiologic studies. A longer term objective is the development of passive and active immunoprophylaxis for these important human pathogens.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00314-09 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Woodchuck Virus: Molecular Biological Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Roger Miller, Ph.D	Senior Staff Fellow	LID, NIAID
Others:	Cathy Chung, Ph.D.	Senior Staff Fellow	LID, NIAID
	Hong-shu Chen, M.D.	Visiting Fellow	LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS (if any)

Division of Molecular Virology & Immunology, Georgetown University, Washington, DC (Gerin); New York State College of Veterinary Medicine (Tennant)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	1.7	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The woodchuck hepatitis virus (WHV) is taxonomically and serologically related to the hepatitis B virus (HBV). Infection with each of these viruses is associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts and these associations appear to be etiological in nature. Thus, WHV infection of woodchucks provides a relevant and convenient model for understanding HBV infections of humans. The following experiments were successfully completed: (1) Recombinant WHV DNA was transfected into the liver of susceptible woodchucks and acute and chronic WHV infections were produced. The complete nucleotide sequence of the infectious WHV DNA was determined. (2) Serum pools were prepared from chronically infected animals to be used in future experiments. The virus present in these pooled sera represents the equivalent of "plaque purified" virus in our model system. (3) The mutation rate for WHV replication was estimated to be $<2 \times 10^{-4}$ changes per genome per year, which is lower than the mutation rate of other viruses whose polymerase also lacks an associated proof-reading function. (4) A primary woodchuck hepatocyte tissue culture system was developed for use in transfection experiments.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00317-09 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biology of Non-A, Non-B Hepatitis (NANBH) Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Stephen M. Feinstone, M.D.	Medical Officer	LID, NIAID
Others:	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS (if any)

Blood Bank, NIH Clinical Center, Bethesda, MD (Alter, Shih, Esteban); CH, LMG, NIH, Bethesda, MD (Dawid, Sargent); Nihon University School of Medicine, Tokyo, Japan (Shimizu); Cetus Corporation, Emeryville, CA (Sninsky)

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Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1989-90

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00370-08 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Acquired Immune Deficiency Syndrome (AIDS)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID
Others:	Simoy Goldstein, Ph.D.	Expert	LID, NIAID

COOPERATING UNITS (if any)

LIG, NIAID, NIH, Bethesda, MD (Fauci, Koenig); Georgetown University (Formsgard, Gerin, Hirsch, Johnson, London, Olmsted); Delta Primate Center (Murphy-Corb); Soutwest Foundation (Allen); NCI (O'Brien); Florida Game Commission (Roelke)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	1.7	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A new effort concentrating on animal models of AIDS was undertaken in August, 1987. Two model systems were established: (1) a primate model using the various simian immunodeficiency viruses (SIV); and (2) a feline model using the feline immunodeficiency virus (FIV). These viruses belong to the lentivirus subfamily of retroviruses and induce chronic persistent infection and immunodeficiency in their respective hosts. The SIVs are more or less genetically related to the human immunodeficiency viruses (HIV-1 and HIV-2), while FIV is much less closely related to human or primate viruses based on lack of antigenic cross-reactivity and limited sequence homology.

Challenge pools of FIV and several SIVs have been prepared and are undergoing infectivity titration in animals. Disease (simian AIDS) has been produced by SIV and cats have been infected by FIV, but to date minimal disease has been detected. Both FIVs and SIVs have been cloned and sequenced and their sequences compared with those of other related lentiviruses.

Genetic drift of SIV over a 12 month interval was documented in monkeys experimentally infected with a molecular clone of SIV_{SM}. Significant variation was detected in the envelope glycoprotein gene but not in the integrase gene. SIV isolates from healthy infected monkeys accumulated multiple in-frame stop codons in the env gene, whereas isolates from immunodeficient animals did not have such mutations. An inactivated SIV vaccine prepared by psoralen/UV treatment induced SIV envelope glycoprotein antibodies after a single inoculation.

Significant sequence diversity in the env gene was detected among FIV isolates from different geographic locations. Also, serologic evidence of infection with FIV was detected with high frequency among free-ranging, wild caught felids such as Florida panthers (*Felis concolor*).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00406-07 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Structure of HAV: Genomic Sequence and Organization

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Suzanne U. Emerson, Ph.D.	Microbiologist	LID, NIAID
Others:	Czeslaw Wychowski, Ph.D.	Visiting Associate	LID, NIAID
	Stephen M. Feinstone, Ph.D.	Medical Officer	LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS (if any)

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Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1989-90

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00440-06 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Construction of Full Length Hepatitis A Virus cDNA for Transfection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Suzanne Emerson, Ph.D.	Microbiologist	LID, NIAID
Others:	Stephen M. Feinstone, M.D.	Medical Officer	LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS (if any)

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Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1989-90

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00530-03 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular and Computer Analysis of the Hepatitis B Virus Genome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Roger H. Miller, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Cathy Chung, Ph.D.	Senior Staff Fellow	LID, NIAID
	Rosina Girones, Ph.D.	Visiting Fellow	LID, NIAID
	Shuichi Kaneko, M.D.	Visiting Fellow	LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS (if any)

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Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to analyze the hepatitis B virus (HBV) genome using computer and molecular techniques. Using computer analysis we determined the location of the X transcript promoter element and extended our previous observations that suggest an evolutionary relationship between hepadnaviruses and retroviruses. Using molecular techniques we mapped the sequence heterogeneity of HBV genomes from virus present in the serum of a patient chronically infected with HBV. These HBV genomes exhibited an unexpectedly high degree of sequence heterogeneity.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00555-02 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Detection of Hepatitis B Virus DNA Using the Polymerase Chain Reaction Assay

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Roger H. Miller, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Shuichi Kaneko, M.D.	Visiting Fellow	LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS (if any)

Liver Diseases Section, National Institutes of Diabetes and Digestive and Kidney Diseases (Di Bisceglie, Hoofnagle)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Standard methods of virus DNA detection using the polymerase chain reaction (PCR) can be time consuming and involve multiple steps during which contamination with exogenous DNA often occurs. Therefore, we developed a simplified method for detecting hepatitis B virus (HBV) DNA in serum. The main advantages of this method are that it can be performed rapidly, it consists of only a few steps, and has a false positive rate of <0.1% in our laboratory. In testing serum from 84 human patients, we found HBV DNA in all patients who had HBsAg and hepatitis B e antigen (HBeAg) in serum and in 64% of the patients who had HBsAg, but not HBeAg, in serum. Also, 3 of 11 patients who were chronic HBV carriers and who subsequently lost HBsAg were found to have HBV DNA in serum. In contrast, all patients who lost HBsAg after acute HBV infection or those classified as having non-A, non-B hepatitis were negative for HBV DNA. Thus, the modified PCR technique is a sensitive and rapid method for detecting HBV DNA-containing virions in serum.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00569-01 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Determination of Genetic Markers of Virulence & Adaptation to Cell-Culture of Hepatitis A Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Suzanne U. Emerson, Ph.D.	Microbiologist	LID, NIAID
Others:	Sergei Tsarev, Ph.D.	Visiting Fellow	LID, NIAID
	Valeria Tedeschi, Ph.D.	Visiting Scientist	LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS (if any)

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Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A single-base deletion in the middle of the wild-type hepatitis A virus (HAV) HM-175 cDNA clone was discovered. This deletion resulted in a frame-shift mutation which probably accounted for the lack of infectivity of this full length cDNA clone. This deletion was corrected by oligonucleotide directed mutagenesis yielding a genome that was infectious when the 5' non-coding region of the cell culture adapted mutant was substituted for the corresponding wild type sequence.

Analysis of the growth of chimeric HAV viruses differing only in the 5' non-coding region established that mutations in this region can impart a host range effect; viruses containing the 5' wild-type sequence were restricted in their replication in the simian CV-1 cell line compared to viruses containing the 5' sequence of the cell-culture adapted mutant. Thus, both the 5' non-coding region and the 2B/2C region of the viral genome contain important determinants of host range and replicative capacity *in vitro*.

Greater than 95% of the sequence of a simian isolate of HAV was determined by direct sequencing of the polymerase chain reaction product obtained using specific primers and a homogenate of the liver of an infected African green monkey. The sequence differs substantially from that of any known human isolate of HAV. Preliminary studies demonstrated that this virus grows very efficiently *in vitro* in primary African green monkey kidney cells or in a continuous cell line of fetal rhesus kidney cells.

The polymerase chain reaction was used to determine the sequence of the 5' non-coding and 2B/2C regions of the master seed pools of passage 21 and passage 32 lots of HAV candidate vaccine virus.

A continuous simian cell line stably transformed with a plasmid containing the P2 region from the Sabin 1 strain of poliovirus was shown to support growth of HAV. In addition, the infectivity of the cell-adapted HAV mutant was increased 10 to 100-fold when assayed in the poliovirus P2 transformed cell line compared to the non-transfected parental cell line.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00570-01 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of Hepatitis C Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Roger H. Miller, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID
	Patrizia Farci, M.D.	Visiting Scientist	LID, NIAID
	Norio Ogata, M.D., Ph.D.	Visiting Fellow	LID, NIAID

COOPERATING UNITS (if any)

NIH, Japan (Shimizu); Chiron Corporation (Weiner)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hepatitis C virus (HCV) is an important human pathogen that is strongly associated with post transfusion hepatitis as well as sporadic non-A, non-B hepatitis. Recently, HCV cDNA was cloned and its genome sequenced by others. A region of the predicted polyprotein sequence was found to share similarity with a non-structural protein encoded by dengue virus, a member of the flavivirus family. We found, using computer-assisted analysis, that HCV shares an even greater degree of protein sequence similarity with members of the pestivirus group (i.e., bovine viral diarrhea virus and hog cholera virus) which are thought to be distantly related to the flaviviruses. In addition, we found that HCV shares significant protein sequence similarity with the polyproteins encoded by members of the picornavirus-like and alphavirus-like plant virus supergroups. These data suggest that HCV may be evolutionarily related to both plant and animal viruses. PCR amplification was used to study the pathogenesis of HCV infection in patients and experimentally infected primates. An unexpected finding was the observation that HCV replication begins as early as three days following experimental infection of chimpanzees.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00323-09 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Expression of Parainfluenza Type 3 Virus Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Genevieve Mottet	Special Volunteer	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.2	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The intracellular synthesis and maturation of the hemagglutinin-neuraminidase (HN) protein of human parainfluenza virus type 3 (PIV3) was investigated with regard to the kinetics of folding and the formation of homooligomers that constitute the HN virion spike. Folding of the HN protein, as monitored by the acquisition of immunoreactivity with conformation-dependent antibodies and by electrophoretic mobility during nonreducing SDS-PAGE, was found to have a half-time of approximately 15 min. Post-infection cotton rat sera and HN-specific murine monoclonal antibodies (mabs) from a previously-described panel showed little or no reactivity with newly-synthesized, unfolded material, illustrating the conformational nature of the major HN epitopes. The HN protein was shown to form homotetramers by chemical cross-linking and sucrose sedimentation analysis. In all previously studied viral systems, glycoprotein analyzed following a short labeling pulse was present primarily as unfolded monomers which oligomerized only after the monomers had folded into essentially mature conformations. In contrast, for the PIV3 HN protein, newly-synthesized intracellular protein was found exclusively in oligomers which were indistinguishable with regard to sedimentation profile and stability from those of the mature protein. We speculate that oligomerization might be cotranslational and might be related to the type II membrane topography of HN. These studies provide an overview of the time-course of folding and oligomerization of the PIV3 HN protein, demonstrate the conformation-dependence of the major HN epitopes, and illustrate an exception to the previous rule that folding of glycoprotein monomers is a prerequisite to their association into oligomers.

PHS 6040 (Rev. 1/84) GPO 914-918

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00324-09 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Laboratory Studies of Myxoviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
Others:	Mark Perkins, M.D.	Medical Staff Fellow	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS (if any)

University of Rochester (Treanor)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.2	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies were initiated to investigate the genetic stability of the genes of the cold-adapted (*ca*) influenza A/Ann Arbor/60 donor virus that confer attenuation on reassortant viruses. Initially, a single gene reassortant was prepared that contained the PB2 gene of the *ca* donor virus in a background of 7 other genes derived from a human wild type influenza A virus. The PB2 protein is a constituent of the viral polymerase complex that also includes the PB1 and PA proteins. The PB2 gene from the *ca* donor virus specifies a temperature sensitivity (*ts*) phenotype. *ts*+ revertants were selected and analyzed genetically. Four independent *ts*+ revertants developed a suppressor ("second site") mutation in the PA gene. In contrast, sequence analysis indicated that the sequence of the mutant PB2 gene was unaltered. These observations suggest that a suppressor mutation developed in another protein of the polymerase complex (i.e., PA) of the 4 revertants and this mutation corrected the defect in polymerase activity specified by the mutant PB2 gene.

Coding or non-coding mutations in attenuating genes occur infrequently during the process of producing live attenuated influenza A virus vaccine by gene transfer from an attenuated donor virus to a reassortant virus bearing the surface glycoproteins of a wild type human influenza A virus.

The NP and M genes, which are the attenuating genes of the A/Mallard/78 avian influenza A donor virus, are genetically stable after prolonged replication of influenza A virus reassortants in primates or humans.

A single gene reassortant containing the PB2 gene of the attenuated avian influenza A/Mallard/78 donor virus and the other 7 RNA segments derived from the virulent human A/LA/87 (H3N2) virus was shown to be markedly restricted in replication in mammalian cells, but this reassortant replicated efficiently in avian cells. Studies are in progress to determine if this gene constellation will consistently attenuate wild type human influenza A viruses for monkeys and humans.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00325-09 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Respiratory Viruses in Primates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
Others:	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID
	John Treanor, M.D.	Senior Staff Fellow	LID, NIAID
	Kathleen Coelingh, Ph.D.	Expert	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS (if any)

Sema Laboratories, Rockville, MD (Philips); Georgetown University (London); Johns Hopkins University School of Public Health, Baltimore, MD (Prince); Wyeth Laboratories (Hung); Marshall University (Belshe)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	0.4	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A cold-adapted mutant of human parainfluenza virus type 3 (PIV3) cold passage 12 (cp12) was attenuated, immunogenic, and protective in chimpanzees. These observations form the basis for evaluation of this mutant and its more attenuated progeny (cp18 and cp45) in humans, including seronegative infants and children.

Vaccinia recombinant viruses were constructed that expressed Il-2 and the influenza hemagglutinin (HA). It was found that IL-2 expression significantly reduced the skin lesions induced by the live vaccinia virus recombinant in primates. In this manner it was possible to bring about significant attenuation, without reducing the immunogenicity of vaccinia virus-recombinant expressed influenza A virus HA.

Immunization of a chimpanzee with type 4 and type 7 adenovirus-RSV F recombinants administered orally in an enteric coated capsule (one dose of Ad7-F followed by Ad4-F) failed to induce a significant antibody rise to the RSV fusion (F) glycoprotein.

The M or NP gene of the avian influenza A/Mallard/78 donor virus was transferred into a reassortant virus whose other 7 genes were derived from a wild type human influenza H3N2 virus. The M and NP genes, which are the major attenuating genes of the avian influenza A donor virus, were stable genetically after 5 serial passages of these reassortant viruses in monkeys. Furthermore, the reassortant viruses retained their attenuation phenotype following the 5 serial passages in monkeys.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00326-09 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Respiratory Viruses in Volunteers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
Others:	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS (if any)

Flow Labs, Rockville, MD (Potash); Johns Hopkins University, Baltimore, MD (Clements); Vanderbilt University School of Medicine, Nashville, TN (Wright); Marshall University School of Medicine, Huntington, WV (Belshe)

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Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.3	0.9

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Live attenuated bivalent *ca* influenza A virus reassortant vaccine was less immunogenic in the elderly than inactivated influenza A virus vaccine. Little increase in immunogenicity was observed when parenterally administered inactivated vaccine was used in combination with live, bivalent *ca* influenza A virus vaccine administered intranasally. During the course of these studies we observed that the elderly have non-hemagglutination-inhibiting (HAI) antibodies that correlate with resistance to infection with live virus vaccines.

Cold-adapted (*ca*) and avian-human (*ah*) influenza A/Bethesda/85 (H3N2) reassortant virus vaccines were observed to be both safe and immunogenic in seronegative infants and children. However, the *ah*, but not *ca*, reassortant A/Kawasaki/86 (H1N1) virus caused a febrile response in one fourth of seronegative infants and children. Thus, the influenza virus hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins can significantly affect the level of attenuation specified by the six avian influenza A donor virus genes that code for the non-surface viral proteins. In contrast, the 6 genes of the influenza A/AA/6/60 *ca* donor virus that code for the non-surface viral proteins are able to confer a satisfactory level of attenuation upon reassortant viruses bearing either the H1N1 or H3N2 surface glycoproteins of wild type human influenza A virus.

The influenza B/AA/186 *ca* reassortant virus was safe, highly infectious, phenotypically stable, immunogenic, and non-transmissible in seronegative infants.

PHS 6040 (Rev. 1/84)

GPO 914-918

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00327-09 LID

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Characterization of Parainfluenza Type 3 Virus Surface Glycoproteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
Others:	Susan L. Hall, Ph.D.	Staff Fellow	LID, NIAID
	Kathleen Coelingh, Ph.D.	Expert	LID, NIAID
	Robert Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS (if any)

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Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.1	2.1	2.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Neutralizing monoclonal antibodies specific for the fusion (F) glycoprotein of human parainfluenza type 3 virus (PIV3) were used to select neutralization-resistant antigenic variants. Sequence analysis of the F gene of the variants indicated that their resistance to antibody binding, antibody-mediated neutralization or to both was a result of specific amino acid substitutions within the neutralization epitopes of the F1 and F2 subunits. Comparison of the location of PIV3 F neutralization epitopes with the corresponding sites on Newcastle disease virus and Sendai virus indicated that the antigenic organization of the fusion proteins of these other paramyxoviruses is similar. Futhermore, some of the PIV3 F epitopes recognized by syncytium-inhibiting monoclonal antibodies are located in an F1 cysteine cluster region that corresponds to an area of the measles virus F protein involved in fusion activity.

More than 90% of infants or young children who underwent primary infection with PIV3 developed antibodies to 4 of the 6 hemagglutinin-neuraminidase (HN) antigenic sites (including 3 of the 4 neutralization sites), whereas the response to F antigenic sites was of lesser magnitude and varied considerably from person to person. Also, the response to both F and HN antigenic sites was suppressed in infants who possessed maternally-derived serum PIV3 antibodies. The restricted immune response to the F glycoprotein during primary infection and the decreased response to both the F and HN glycoproteins in young infants with maternally-derived antibodies may play a role in the susceptibility of the human infant and young child to reinfection with PIV3.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00345-09 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Laboratory Studies of Respiratory Syncytial Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
Others:	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Gregory Prince, D.D.S., Ph.D.	NIH Special Volunteer	LID, NIAID
	Peter Collins, Ph.D.	Senior Staff Fellow	LID, NIAID
	Mark Connors, M.D.	Medical Staff Fellow	LID, NIAID

COOPERATING UNITS (if any)

USUHS, Bethesda, MD (Hemming); Praxis Biologics, Rochester, NY (Paradiso); Georgetown University (Jensen); Upjohn Company, Kalamazoo, MI (Wathen)

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SECTION

Respiratory Viruses Section

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.8	1.2	2.6

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Enhanced pulmonary pathology was observed in cotton rats immunized with formalin-inactivated respiratory syncytial virus (RSV) or purified RSV fusion (F) protein and challenged intranasally with RSV 3 or 6 months later. This constitutes the first evidence that immunization with the F glycoprotein alone can induce a potentiated pathological response to subsequent RSV infection. Enhanced pulmonary pathology was not observed previously when animals immunized with purified RSV F were challenged with RSV one month later. These observations indicate the importance of the interval between immunization and subsequent virus infection in the occurrence of enhanced pulmonary pathology. A similar situation was observed almost 25 years ago with the licensed inactivated measles virus vaccine and thus, caution should be exercised in the evaluation of a purified RSV F glycoprotein vaccine in humans. Also, passively acquired RSV F antibodies can suppress the neutralizing antibody response of cotton rats to purified RSV F glycoprotein. With lower doses of F (0.2 ug to 1.7 ug) there was a 7 to 8 fold suppression, while with high doses of F (5 to 15 μg) suppression was significantly less, i.e., 2 fold.

The efficiency of topical immunotherapy for RSV infection was significantly increased by two modifications of previous methodology. First, a mixture of RSV F monoclonal antibodies directed at the major conserved neutralization epitopes on this glycoprotein was highly effective in topical immunotherapy of RSV infection in the cotton rat. Second, delivery of RSV antibodies directly into the lungs in a small particle aerosol (< 2 mu) was also effective therapeutically. The use of monoclonal antibodies should decrease the amount of IgG required for therapy by 2 orders of magnitude. In other studies in cotton rats, parainfluenza virus type 3 (PIV3) antibodies were shown to be protective as well as therapeutic. Thus, usefulness of topical immunotherapy is not limited to RSV. It is likely that this approach will prove to be effective for other respiratory viral pathogens whose pathogenic effects are also limited to the cells that line the lumen of the lower respiratory tract.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00368-08 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Analysis of Respiratory Syncytial Virus (RSV) Genome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	David S. Stec, Ph.D.	NCR Fellow	LID, NIAID
	Michael A. Mink, Ph.D.	IRTA	LID, NIAID

COOPERATING UNITS (if any)

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Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.7	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A complete sequence was obtained for the 6578-nucleotide L gene, the 155-nucleotide 5'-trailer region and the 44-nucleotide leader region of RSV genomic RNA (vRNA) of strain A2 of subgroup A. This completes the sequence analysis of the 15,222-nucleotide RSV vRNA.

cDNAs have been constructed for the *in vitro* syntheses of synthetic vRNA-like molecules. These "vRNAs" contain a marker gene (chloramphenicol acetyltransferase) under the control of RSV transcriptive signals and flanked by the RSV 3' and 5' vRNA sequences that are thought to contain promoters that direct transcription and replication. We are exploring methods for introducing these synthetic vRNAs into RSV-infected cells such that they will associate with viral proteins and be transcribed and replicated.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00372-08 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

RSV Proteins: Roles in Host Immunity and Immunoprophylaxis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Geoffrey Cole, Ph.D.	IRTA	LID, NIAID
	Mark Connors, M.D.	Research Associate	LID, NIAID

COOPERATING UNITS (if any)

LVD, NIAID, NIH (Moss, Buller); Johns Hopkins University School of Public Health, Baltimore, MD (Prince); Wyeth Laboratory, Rancor, PA (Davis, Hung); the Upjohn Company, Kalamazoo, MI (Nicholas).

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are investigating approaches for utilizing recombinant DNA techniques in developing vaccines for human respiratory syncytial virus (RSV). Vaccinia virus recombinants expressing the RSV F or G glycoprotein were highly immunogenic and effective in inducing resistance to RSV infection in rodents and monkeys but, surprisingly, these recombinants were not protective in chimpanzees.

To investigate the participation of individual RSV proteins in host immunity, recombinant vaccinia viruses were constructed which individually expressed seven additional RSV genes, namely the 1C, 1B, N, P, M, 22K M2, and SH genes, and construction of a recombinant for the remaining gene, L, is underway. These recombinants are being evaluated for immunogenicity, protective efficacy, and possible disease potentiation in rodents. To date, the SH and N proteins did not induce significant protective immunity, in contrast to the high levels of protective immunity elicited by the F and G glycoproteins. The 22K M2 protein was found to be the major viral target antigen for RSV-specific murine cytotoxic T lymphocytes, with F and N being secondary targets, but the importance of cytotoxic T cells in immunity or disease enhancement remains to be defined.

The use of adenovirus as a viral vector offers several potential advantages for RSV immunoprophylaxis, and previously a type 5 recombinant expressing the RSV F protein was constructed and shown to induce high levels of protective immunity in cotton rats. This work is being extended by constructing parental adenovirus vectors containing various engineered insertion sites which are designed to minimize alteration of adenovirus gene expression and replication. These vectors will be used to construct recombinants expressing the RSV F protein that will be evaluated for (1) replicative capacity, (2) alteration in pathogenesis during immunization, (3) immunogenicity and protective efficacy of the expressed RSV protein, and (4) the possibility that immunization potentiates pulmonary pathology during subsequent infection with RSV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00497-04 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of the Fusion Glycoprotein of Respiratory Syncytial Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kathleen Coelingh, Ph.D. Senior Staff Fellow LID, NIAID

COOPERATING UNITS (if any)

Food and Drug Administration (Beeler)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1989-90

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00498-04 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synthesis, Processing and Functions of the Proteins of Human RSV

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	David S. Stec, Ph.D.	NRC Fellow	LID, NIAID

COOPERATING UNITS (if any)

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Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	0.7	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Analysis of the respiratory syncytial virus (RSV) mRNAs by cDNA cloning, sequencing and related techniques has identified ten different mRNAs, and to date a single encoded polypeptide has been identified for each. We are interested in (i) continuing the analysis of the viral mRNAs and their translational open reading frames (ORFs) to detect possible additional mRNAs and polypeptides and to learn more about the details of RSV gene expression, (ii) defining the extent of sequence diversity among RSV isolates, and (iii) analyzing the individual RSV proteins to determine their intracellular and virion locations and their functions. We have continued the analysis of the 1A protein (whose name has been changed to SH [small hydrophobic] to be consistent with the nomenclature for simian virus 5 and mumps virus). The SH protein was shown to be an integral membrane protein that is processed by a complex intracellular pathway that results in two nonglycosylated and two glycosylated species. The processing pathway and structural differences between the different forms have been shown to be conserved between the two RSV antigenic subgroups. Experiments are continuing to determine the membrane orientation, site of glycosylation, and the oligomeric status of the different forms of the SH protein. In other work, a complete nucleotide sequence was determined for the 22K gene of strain 18537. Comparison with the previously-determined strain A2 sequence revealed a high level (92%) of amino acid conservation for the 22K (M2) protein and identified a second, conserved ORF whose protein product remains to be identified. The high level of conservation of the 22K protein is of significance because it has been shown to be the major target antigen for RSV-specific cytotoxic T cells from infected mice (accompanying report).

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00458-05 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Variations Among Dengue Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ching-Juh Lai, Ph.D. Head, MVB Section LID, NIAID

COOPERATING UNITS (if any)

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Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1989-90

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00459-05 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of Dengue Virus Proteins Using Baculovirus as a Vector

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
Others:	Robert Chanock, M.D.	Chief	LID, NIAID
	Ruhe Men, M.D.	Special Volunteer	LID, NIAID
	Michele Pethel	Microbiologist	LID, NIAID
	Yi-Ming Zhang, M.D.	Visiting Associate	LID, NIAID

COOPERATING UNITS (if any)

WRAIR, Washington, DC (Summers, Eckels, Dubois); Rochester General Hospital, University of Rochester, Rochester, NY (Schlesinger)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	0.8	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

INACTIVE 1989-90

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00476-05 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Engineering the Genome of Dengue Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
Others:	Hiroyuki Hori, M.D.	Visiting Associate	LID, NIAID
	Michael Bray, M.D.	Senior Staff Fellow	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS (if any)

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Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

One of our major research objectives for the dengue viruses has been the construction of full-length dengue cloned DNA that could be used for the production *in vitro* of full-length RNA transcripts able to initiate dengue virus infection following transfection of permissive cells in culture. Our initial strategy was to use the pBR322 plasmid vector to clone full-length dengue DNA from two fragments, each containing approximately one-half of the dengue genome sequence. The full-length dengue virus cDNA was placed under the control of an SP6 polymerase promoter and a unique Asp 718 cleavage sequence was inserted at the 3'-end of the dengue sequence to be used for producing a run-off transcript. Asp 718 linearized DNA was then used as a template for *in vitro* transcription of RNA using SP6 polymerase. m^7G capped GTP was added to the transcription reaction to produce full-length dengue RNA transcripts containing a cap structure at the 5' end. Transfection of LLCMK2 cells with the transcription products yielded infectious dengue virus as indicated by the appearance of dengue antigens in a majority of cells. Progeny dengue virus was recovered at a titer of 10^5pfu/ml from the transfected cell lysate. Treatment of the transcription products with DNase did not affect infectivity, while RNase treatment completely abolished their infectivity. Formal proof for the infectivity of the RNA transcripts was provided by engineering two silent mutations into the dengue cDNA and demonstrating the presence of these mutations in dengue virus recovered from cells transfected with the mutant RNA transcripts. Experiments are underway using the full-length dengue cDNA to engineer mutations at strategic sites in the dengue viral genome. Subsequently, infectious dengue virus containing the mutations introduced into dengue cDNA will be evaluated for various biological and immunological properties *in vitro* as well as alteration in virulence for experimental animals. This strategy may yield attenuated mutants that could be used in a safe, effective, live virus vaccine.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00477-05 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Analysis of Dengue Virus Envelope Glycoprotein and its Immunogenicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ruhe Men, M.D.	Special Volunteer	LID, NIAID
Others:	Michael Bray, M.D.	Senior Staff Fellow	LID, NIAID
	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS (if any)

WRAIR, Washington, DC (Eckels)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.4	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The antigenic structure of dengue envelope (E) glycoprotein was investigated with the goal of increasing its immunogenicity. Recombinant vaccinia viruses were constructed that contained dengue cDNA coding for C-terminally truncated E that ranged in length from 9% to 99% of the N-terminal sequence. Overall antigenicity of the E products was analyzed by radio-immunoprecipitation using dengue hyperimmune mouse ascitic fluid (HMAF) or an E peptide antiserum. With the exception of 59%E, only E constructs greater than 79% were able to bind HMAF efficiently. Arg at position 392 in the dengue type 4 E sequence immediately following the 79%E C-terminus appeared to be critical for proper conformation and antigenic structure required for efficient binding by HMAF. Full-length E was retained intracellularly, and only truncated E ranging from 59% to 81% in length was secreted extracellularly. Only 79%E-RKG (that possessed the 3 additional amino acids immediately downstream of 79%E) was expressed in high concentration on the surface of recombinant virus-infected cells presumably being inserted into the plasma membrane by a C-terminal membrane anchor that included a 20 amino acid hydrophobic transmembrane domain and a short charged amino acid cytoplasmic domain. Studies in mice indicated that only truncated E's that were recognized efficiently by HMAF induced a high level of resistance to dengue virus encephalitis. Among the truncated E's that were able to bind HMAF efficiently only 79%E-RKG was expressed in high concentration on the cell surface. Significantly, only 79%E-RKG induced an appreciable E antibody response suggesting that cell surface expression was responsible for its enhanced immunogenicity. Finally, passive immunization studies in mice involving serum transfer indicated that serum antibodies to E played a major role in the complete or nearly complete resistance to dengue virus challenge induced by certain vaccinia virus-truncated E recombinants.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00499-04 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of Selected Dengue E Protein Sequences by Recombinant Vaccinia Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael Bray, M.D.	Senior Staff Fellow	LID, NIAID
Others:	Lewis Markoff, M.D.	Medical Officer	LID, NIAID
	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS (if any)

LVD, NIAID, NIH (B. Moss)

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Laboratory of Infectious Diseases

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.4	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recombinant vaccinia viruses expressing dengue type 4 virus (D4) and dengue type 2 virus (D2) proteins from cloned DNA were constructed for evaluation in experimental immunoprophylaxis. Previously we showed that mice immunized with recombinant vaccinia viruses which expressed the D4 pre-M and E glycoproteins, or E alone, were protected against homotypic dengue virus encephalitis. However, an attempt to prevent dengue viremia in rhesus monkeys by immunization with a vaccinia virus recombinant expressing pre-M, E and non-structural protein NS1 was unsuccessful. Mice immunized with an E recombinant developed a low level of neutralizing antibodies, but antibodies were not detectable by radio-immunoprecipitation. Recently, recombinants expressing pre-M and E of the S-1 candidate live vaccine strain of D2 virus induced solid protection in mice against challenge with 100 LD_{50} of D2 strain New Guinea C. A recombinant expressing D2 E alone induced only partial protection against D2 challenge, in contrast to complete homotypic protection provided by a D4 E recombinant. A recombinant which expressed the N-terminal 79% of D2 E, that is secreted into the medium, induced a strong antibody response to E in immunized mice and provided solid protection against homotypic virus challenge equivalent to that provided by vD2 pM-E. Prior infection with a vaccinia recombinant expressing apparently authentic E did not prime the antibody response to subsequent immunization with dengue virus or baculovirus expressed E. An analysis of vaccinia recombinants that expressed E fusion proteins, in which the N-terminal half consisted of D4 E and the C-terminal half of D2 E, or vice-versa failed to identify the region of the E molecule responsible for eliciting a type specific protective immune response.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00500-04 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Processing and Immunogenicity of Dengue Type 4 Virus Nonstructural Protein NS1

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Barry Falgout, Ph.D.	Staff Fellow	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
	Michael Bray, M.D.	Senior Staff Fellow	LID,NIAID

COOPERATING UNITS (if any)

Rochester General Hospital (Schlesinger)

LAB/BRANCH

Laboratory of Infectious Diseases

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Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We studied the processing of dengue virus nonstructural protein NS1 by *in vitro* transcription/translation, and *in vivo* as expressed by recombinant vaccinia virus. *In vitro*, the full length NS1-NS2A precursor was made, and was translocated into dog pancreas microsomal membranes and glycosylated, but NS1/NS2A cleavage did not occur. *In vivo*, brefeldin A blocked secretion of NS1 but did not inhibit NS1/NS2A cleavage. Taken together, these results suggest that NS1/NS2A cleavage occurs in the Golgi, or in a compartment between the ER and the Golgi. We constructed vaccinia recombinant viruses expressing chimeric preM-NS1(3/8)-NS2A proteins, containing only the 3 or 8 C-terminal amino acid residues of NS1. We observed that the chimera with 8 residues of NS1 was cleaved at the NS1-NS2A junction. Thus, the only portion of NS1 required for NS1/NS2A cleavage is the region containing the 8 C-terminal amino acids. Results with analogous NS5-NS1(3/8)-NS2A chimeras showed that these proteins were partially cleaved to about the same extent. These results suggest that the cleavage efficiency and NS1 target size are both reduced when the NS1-NS2A junction is not translocated into the ER.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00501-04 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of the Processing of Dengue Viral Glycoproteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Lewis J. Markoff, M.D.	Medical Officer	LID, NIAID
Others:	Barry Falgout, Ph.D.	Staff Fellow	LID, NIAID
	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS (if any)

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Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Processing of the dengue virus polyprotein is initiated by cleavage of the capsid protein from the pre-membrane protein (pM) at a site downstream from the pM signal sequence presumably recognized by the enzyme signal peptidase. Cleavage of pM from the N-terminus of the envelope glycoprotein (E) also appeared to be mediated by signal peptidase. Requirement for autoproteolytic activity of the dengue capsid protein was not detected. In order to extend these observations RNA transcripts prepared from wild type (wt) and mutant dengue DNA encoding the N-terminal 1,040 or 691 amino acids of the polyprotein were translated *in vitro* in rabbit recitulocyte or wheat germ extracts in the presence or absence of added dog pancreatic microsomal membranes. Results have not been interpretable due to internal initiation of translation at multiple sites and/or failure of commercially purchased membranes to mediate cleavage. Capping of RNA transcripts and immune-precipitation of cell-free translation products with rabbit antibodies to peptides representing the N-termini respectively of capsid, pM, and E currently under study may solve these problems. In addition, five mutations were cloned into a 4kb fragment of dengue DNA encoding capsid, pM, E, NS1, and NS2A present in the vector pSC11 to facilitate recombination into vaccinia virus. These mutations included: (1) a deletion of a trypsin-like potential cleavage site (Arg-Lys-Arg; residues 97-99) which precedes the pM signal; (2) a substitution mutation of the pM signal in which residues 109 and 111 have been converted to hydrophilic Lys and Arg, respectively; (3) deletion of the upstream hydrophobic segment in pM (246-263); (4) alteration of Arg (residue 264) to Phe-Phe-Val; (5) deletion of the downstream hydrophobic segment in pM (265-279). Analysis of phenotypes of mutant vaccinia virus recombinants is not complete. Mutation (2) appears to abrogate capsid-pM cleavage; mutation (3) results in partial inhibition of pM-E cleavage. Mutation (5) completely inhibits pM-E cleavage. Further studies of these mutants and of the fate of capsid in vaccinia virus and dengue virus infected cells are in progress.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00502-04 LID

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Antigenic Analysis of the Dengue Virus Envelope Glycoprotein (E) Using Synthetic Peptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Lewis J. Markoff, M.D.	Medical Officer	LID, NIAID
Others:	Peter Braverman	Student	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS (if any)
FDA (Berkower); Torrey Pines Institute for Molecular Studies, La Jolla, CA (Houghton)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We attempted to define the immune response to the dengue type 4, strain 814669, envelope glycoprotein (E) in Balb/c (H-2^d) and CBA/N or Balb/K (H-2^k) mice using 38 synthetic 15-amino-acid peptides that span the E sequence (previously described). An *in vivo* assay in which mice were immunized with peptide(s) followed by infectious dengue virus and an *in vitro* assay of proliferation in response to peptides of T lymphocytes isolated from virus immunized mice were conducted. Peptides 39 (amino acids 137 to 151) and 41 (158-172) elicited high titer peptide and dengue 4 virus binding antibodies in Balb/c mice, as measured by ELISA. Peptides B (30-55) and 53 (368-382) elicited IgG class peptide-binding antibodies only. Peptide 54 (381-395) appeared to contain a T cell epitope only. In CBA/N or Balb/k mice, peptide 67 (17-30) elicited a response comparable to that of peptides 39 and 41 in Balb/c mice. Peptides 53 and 72 (233-246) elicited high titer IgG peptide antibodies only. Although the T cell proliferation assay has not yet worked well in H-2^k mice, it is apparent that all these peptides contain T cell epitopes as defined in that assay. Peptides 67, 52 (356-369), and 53 were to varying degrees immunogenic in both H-2^d and H-2^k mice. Additional data show that priming with a given peptide in the *in vivo* assay could result in an anamnestic response to linear epitopes not included on the priming peptide, shortly after the mice were given infectious dengue 4 virus intraperitoneally.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00531-03 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional Analysis of Dengue Nonstructural Proteins NS2B and NS3

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Barry Falgout, Ph.D.	IRTA	LID, NIAID
Others:	Michele Pethel	Microbiologist	LID, NIAID
	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS (if any)

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Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have employed vaccinia virus as a vector to express various portions of the dengue type 4 virus genome in order to study proteolytic processing in the nonstructural protein (NS) region. This study was carried out in part to test the processing model proposed recently predicting that NS3 is a protease based on limited sequence homology to serine proteases. Indirect evidence showing that NS3 can act *in trans* to cleave NS2A/NS2B and NS2B/NS3 was obtained from co-infection experiments using vNS2A-NS2B and vNS2A-ΔNS2B(10)-30%NS3 or vNS2A-ΔNS2B(20)-30%NS3 in which full length NS2B was produced. However, co-infections using vNS2A-NS2B or vNS2A-NS2B-12%NS3 and vNS3 or v30%NS3 did not demonstrate efficient trans-acting activity of NS3. The reason for this discrepancy is not known. Our results demonstrate that NS2B is also required for these NS cleavages. NS2B acts *in trans* to facilitate NS2B/NS3 and NS2A/NS2B cleavage. Thus, both NS2B and NS3 together appear to be required for a protease activity that cleaves NS2A/NS2B and NS2B/NS3. In addition, the protease activity presumably is also responsible for the NS3/NS4A and NS4B/NS5 cleavages. Our results also showed that NS2B is needed for the NS3/NS4A cleavage. This activity of NS2B is either *cis*-acting or very inefficiently trans-acting.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00556-02 LID

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Epitopes on Dengue Virus Nonstructural Protein NS1 that Mediate Protection Against Dengue

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Hiroyuki Hori, M.D.	Visiting Associate	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS (if any)
Rochester General Hospital, University of Rochester, Rochester, NY (Schlesinger)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.0	0.1

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The length and sequence of amino acids at the NS1-NS2A junction of the dengue virus polyprotein that are required for specific cleavage effected by the *cis*-acting function of NS2A were identified by deletion analysis. Recombinant DNA sequences of NS1-NS2A, each containing a deletion in NS1 followed by a sequence of 3-20 amino acids at the C-terminus of NS1 preceding the cleavage site, were constructed and expressed using vaccinia virus as a vector. The NS1 product of recombinant vaccinia virus infected cells was immunoprecipitated and analyzed by SDS-polyacrylamide gel electrophoresis. The occurrence of cleavage between NS1-NS2A was indicated by the appearance of shortened NS1. Failure to cleave this site yielded a large NS1-NS2A fusion protein. This analysis indicated that a minimum length of 8 amino acids at the NS1 C-terminus preceding the NS1-NS2A juncture is required for cleavage to take place. Comparison of this 8 amino acid sequence of the NS1 C-terminus of dengue type 4 virus with the analogous sequences of 12 other flaviviruses suggests that the consensus cleavage site sequence is as follows:

```
Amino acid position with respect to cleavage site
                                                   Cleavage
 -8         -7    -6    -5    -4    -3    -2    -1    ↓
Leu/Met - Val - Xaa - Ser - Xaa - Val - Xaa - Ala
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PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00557-02 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cross-reactive Antibodies to Clotting Factors in Patients Infected with Dengue Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Lewis J. Markoff, M.D. Medical Officer LID, NIAID

COOPERATING UNITS (if any)

WRAIR, Bangkok, Thailand (Innis); Torrey Pine Institute for Molecular Biology, San Diego, CA (Houghten)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The four serotypes of dengue virus (a mosquito-borne flavivirus) cause an acute febrile illness that occasionally results in hospitalization for treatment of hemorrhage (dengue fever; DF) or hemorrhage plus plasma leakage resulting in hypovolemia (dengue hemorrhagic fever; DHF). Hospitalization for these complications usually occurs during a second heterotypic infection, suggesting a role for dengue antibodies in pathogenesis. The major antigen of the virus, against which neutralizing, hemagglutination-inhibiting, and flavivirus cross-reactive antibodies are directed, is the envelope glycoprotein (E). Computer analysis revealed a 20 residue region of similarity in amino acid sequence between the dengue type 4 E and a family of clotting factors, including plasminogen, the prime mediator of fibrinolysis. Using synthetic peptides in ELISA, E antibodies that potentially bind plasminogen were detected in 75% of 40 Thai patients acutely infected with dengue virus type 1, 2, 3, or 4. Plasminogen cross-reactivity of dengue antibodies was shown to be specific for the related sites in E and plasminogen. The dengue E sequence with similarity to plasminogen is largely conserved within the currently known flavivirus E sequences. However, 15 Thai patients hospitalized for illness caused by Japanese encephalitis (JE) virus (a flavivirus not associated with hemorrhage) did not develop plasminogen cross-reactive antibodies, and this finding correlated with failure of JE antibodies to bind to the plasminogen cross-reactive site in E. Possible relevance to the pathogenesis of DF and DHF is discussed.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00571-01 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Amino Acid Sequence Motif at the NS1-NS2A Cleavage Junction of Dengue Polyprotein

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michele Pethel	Microbiologist	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Chief, MVB Section	LID, NIAID

COOPERATING UNITS (if any)

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Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Proteolytic processing of the polyprotein encoded by the positive strand RNA viral genome of dengue virus (as well as other flaviviruses) is a prerequisite for viral gene expression. Recent evidence from our Section indicates that a minimum length of 8 amino acids at the NS1 C-terminus preceding the NS1-NS2A junction is required for cleavage to take place. This study was initiated to analyze the amino acid sequence motif that is optimal cleavage of the NS1-NS2A junction during processing of this region of the viral protein. For this purpose, several amino acid substitutions, at position -1 (Gly or Ala), -2 (Thr), or -3 (Val) of the NS1-NS2A junction of the dengue 4 sequence were introduced and the resulting mutant NS1-NS2A was expressed by a recombinant vaccinia virus. Analysis of mutant sequences expressed by a vaccinia virus recombinant indicated that substitution of Ala at position -1, or Val at -3, yielded an uncleaved NS1-NS2A fusion protein suggesting that the amino acids at positions -1 and -3, that are strictly conserved among flaviviruses, are optimal for cleavage of this junction. On the other hand, replacement of Thr at position -2, a position that is not conserved among flaviviruses, had only a slight to moderate negative effect on cleavage. Variation in amino acid sequence at position +1 occurs among flaviviruses. Nonetheless, substitutions at this site resulted in 42% to 90% reduction in cleavage. Work is in progress to introduce amino acid substitutions at other positions (-4 to -8) to extend our analysis of the sequence motif at the NS1-NS2A junction. Also, construction of full length dengue cDNA containing mutations at strategic NS1-NS2A cleavage sites is underway. Mutant viruses recovered from transfected cells will be evaluated with respect to viral growth in cultured cells and virulence in an infected experimental animal host. Ultimately, this analysis may lead to isolation of attenuated dengue virus mutants that have the potential for use in a live virus vaccine for humans.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00572-01 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Processing of Dengue Virus Polyprotein NS3-NS4A-NS4B-NS5 Domain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Annie Cahour, Ph.D.	Visiting Associate	LID, NIAID
Others:	Barry N. Falgout, Ph.D.	Staff Fellow	LID, NIAID
	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS (if any)

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recombinant vaccinia viruses that contain varying lengths of dengue DNA coding for the polyprotein NS2B-NS3-NS4A-NS4B-NS5 domain were constructed in order to examine the cleavage strategy that is utilized for expression of this region. Initially, mono-specific antiserum containing antibodies to dengue NS3 or NS5 were prepared by immunization against the appropriate trp E fusion protein. Cells infected with vaccinia recombinant v(NS2B-NS3-NS4A-NS4B-NS5) produced NS2B, NS3, and NS5, whereas vaccinia recombinant v(NS3-NS4A-NS4B-NS5) produced an uncleaved polyprotein encoded by the dengue DNA sequence in the recombinant. Thus, NS2B is necessary for proper processing of the downstream nonstructural proteins. The defect in cleavage of the NS3-NS4A-NS4B-NS5 polyprotein was complemented during coinfection with v(NS2B-30%NS3) or v(NS2B) plus v(30%NS3) as indicated by the production of NS3 and NS5 identifiable by the appropriate antiserum. It appears that cleavage at the NS3-NS4A and NS4B-NS5 junctions of the dengue polyprotein requires functions provided by *trans* acting NS2B and the N terminal 30% of NS3. Neither NS2B nor NS3 alone is able to mediate these cleavages. Cleavage at the NS4A-NS4B junction appears to be mediated by a specific signalase since a long stretch of hydrophobic sequence precedes the predicted cleavage site. Evidence supporting this cleavage mechanism was also obtained from analysis of the dengue protein products of vaccinia recombinant v(NS4A-NS4B-NS5) infected cells in which cleavage of NS4B-NS5 from the polyprotein was detected. Finally, NS5 antiserum detected a partially cleaved shortened form of NS5 (1-198) in vaccinia recombinant v(NS4B-NS5) or v(NS4B-NS5[1-198]) infected cells. This finding indicates that in the absence of NS2B and NS3, cleavage at the NS4B-NS5 junction can occur at a low level, presumably mediated by an NS4 function. These results suggest that processing of flavivirus nonstructural proteins may utilize more than one cleavage strategy and these cleavage steps are likely complex and highly regulated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00584-01 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of Dengue Pre-M Glycoprotein & its Cleavage Products by Vaccinia Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael Bray, M.D.	Senior Staff Fellow	LID, NIAID
Others:	Ruhe Men, M.D.	Special Volunteer	LID, NIAID
	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS (if any)

WRAIR, Washington, DC (Eckels)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The pre-membrane (pre-M) structural glycoprotein of dengue virus is cleaved during virus maturation to yield a membrane (M) protein, that becomes part of the virion, leaving a residual glycoprotein segment (Mresid). Vaccinia virus recombinants expressing these three proteins of dengue 4 (D4) virus were constructed. Cells infected with the recombinant expressing pre-M (vD4 pM) produced two different forms of the pre-M protein. One form was apparently authentic pre-M, that could be immunoprecipitated by polyvalent antiserum to dengue 4 virus as well as antiserum raised against an N-terminal peptide segment, and the other form was a comigrating protein, detectable only by antipeptide sera, that was linked into oligomeric forms through disulfide bonds. Similarly, cells infected with vD4 Mresid produced two forms of the same protein. These cells secreted the Mresid glycoprotein into the medium; this protein co-migrated with that secreted by D4 virus infected cells. The protein product of vD4 M was not detected by immunoprecipitation. Following immunization almost all mice inoculated with vD4 pM or vD4 M survived subsequent intracerebral challenge with 100 LD50 of homologous D4 virus, while control mice and mice previously inoculated with vD4 Mresid were not protected. Serum transfer experiments provided evidence that protection was mediated by antibodies. The role of pre-M as a protective antigen was further investigated by constructing vaccinia recombinants expressing dengue 2 structural proteins. A recombinant that expressed both D2 pre-M and the envelope (E) glycoproteins induced solid resistance to a 100 LD50 challenge with D2 virus, but recombinants which expressed E or pre-M alone were only partially protective. Protection was enhanced when mice were immunized with both vD2 pM and vD2 E. Thus, M constitutes a third, previously unrecognized, protective antigen of dengue virus that must be taken into consideration during the development of dengue virus vaccines.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00333-09 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Longitudinal Study of Viral Gastroenteritis in Infants and Young Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID
Others:	Kim Y. Green, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
<.1	<.1	<.1

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects [x] (b) Human tissues [ ] (c) Neither

[x] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Rotaviruses have been studied extensively in many parts of the world predominantly by cross-sectional approaches. Such studies have yielded essentially "numerator" data which indicated that rotaviruses are a major cause of diarrheal illness in infants and young children. There has been a paucity of longitudinal viral gastroenteritis studies that yield not only important "denominator" data but also valuable insights into the natural history of a pathogen or illness, with special emphasis on epidemiologic, immunologic and laboratory information. We, therefore, initiated an intensive examination of anal swab and serum specimens obtained during a previous LID long-term longitudinal study (1955-1969) at Junior Village, a welfare institution for homeless, but otherwise normal children. Anal swabs and blood specimens were obtained routinely from the youngest age groups, which ranged from 6 months to about 5 years of age during various periods of the study. Surveillance was carried out by a trained medical staff. Children were housed in cottages according to their age. Rectal temperatures were obtained on each child daily; routinely, a physician examined any child with a rectal temperature of 100.6°F or greater. Careful medical records were kept by the nursing staff on all children. Thus, with this background, we plan to investigate the natural history of rotavirus infections in a longitudinal manner employing newly developed techniques such as serotyping of rotaviruses with VP7 specific monoclonal antibodies and determining the epitope-specific serologic response in sequential sera to determine the scope of homotypic and heterotypic responses. In addition with the availability of rotavirus strains obtained over 20 years ago, it is planned to compare such strains with current isolates at the genetic level.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00339-09 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Isolation and Serotypic Characterization of Human and Animal Rotaviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
Others:	Mitzi Sereno	Microbiologist	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS (if any)

Texas A & M University (Woode); University of Pavia, Italy (Gerna)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Bovine rotavirus strain B223 was shown, by a two-way cross-neutralization assay, to be distinct from any of the 9 established serotypes. In addition, approximately 75% of the VP7 gene has been sequenced and available data are consistent with its designation as a new serotype.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00340-09 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Experimental Studies of Various Rotaviruses and Their Reassortants in Animals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
Others:	Mitzi M. Sereno	Microbiologist	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS (if any)

Ohio Agricultural and Development Center, Ohio State University, Wooster, OH (Saif)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.2	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Continued efforts have been made to delineate the genetic mechanisms underlying virulence of rotavirus in the newborn gnotobiotic piglet model. In addition to the gene encoding rotavirus outer capsid protein VP4 or VP7, the tenth gene appears to play an independent role in determining the virulence of rotavirus in the porcine model. Elucidation of the constellation of genes required for rotavirus virulence may have important implications for the development of effective rotavirus vaccine strategies.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00341-09 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation of Live Attenuated Rotavirus Vaccines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID
Others:	J. Flores, M.D.	Visiting Scientist	LID, NIAID
	K. Y. Green, Ph.D.	Senior Staff Fellow	LID, NIAID
	M. Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID
	Y. Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
	R. M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS (if any)

Instituto de Biomedicina, Caracas, Venezuela (Pérez-Schael); University of Rochester (Dolin, Madore, Christy); Johns Hopkins University (Midthun, Clements, Halsey, Black); University of Tampere (Vesikari, Ruuska), Vanderbilt University (Wright)

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.6	0.9

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Rotaviruses are the single most important etiologic agents of severe diarrheal illness of infants and young children worldwide. Thus, there is an urgent need for a rotavirus vaccine that can prevent severe rotavirus diarrhea during the first two years of life when this illness is most serious. The "Jennerian" approach to vaccination, which involves use of a live vaccine virus strain derived from a non-human host, has been evaluated in clinical trials of (i) a bovine rotavirus by others, or (ii) a rhesus rotavirus (RRV) strain by the Epidemiology Section, LID. This strategy has had limited success because serotype-specific immunity against all 4 epidemiologically important human rotavirus serotypes (specified by outer capsid protein VP7) could not be achieved consistently in infants less than 6 months of age who had not undergone prior rotavirus infection. Highly effective protection was observed when the simian RRV vaccine (VP7 serotype 3) was used to immunize young infants who were later exposed to human rotavirus strains of the same VP7 serotype. However, in other trials in which rotavirus with a VP7 serotype 1 predominated in the community, vaccine efficacy was variable. As a consequence, most recent clinical trials have employed a "modified Jennerian" approach in which a quadrivalent vaccine of broader antigenic coverage (that includes viruses of VP7 serotype 1, 2, 3, and 4) was used for immunization. This vaccine contains RRV (serotype 3) and reassortant rotaviruses containing 10 RRV genes and a single human rotavirus gene that encodes VP7 serotype 1, 2, or 4 specificity.

In addition, a naturally attenuated human rotavirus strain, M37, with a VP7 specificity of serotype 1 and a unique VP4 specificity shared by other neonatal strains belonging to VP7 serotype 1, 2, 3, or 4, is also under evaluation. Phase 1 trials of the human rotavirus M37 live vaccine have been completed successfully. The M37 rotavirus vaccine was shown to be safe and immunogenic in a clinical study involving 150 young infants (2-4 months of age) and as a consequence phase 2 efficacy trials are now in progress.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00342-09 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Gastroenteritis Viruses by Electron Microscopy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID

COOPERATING UNITS (if any)

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Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The electron microscope has been a mainstay in the study of gastroenteritis viruses. Two major groups of gastroenteritis viruses--the 27nm Norwalk virus and the 70nm human rotaviruses--were discovered at NIH and in Australia, respectively, (as well as the hepatitis A virus at NIH, in collaboration with the Hepatitis Section) with the use of the electron microscope. It is remarkable that in this era of tissue culture virology, these agents were discovered initially without the use of an *in vitro* tissue culture system, since they could not be grown directly from clinical specimens in cell culture. We have applied the term, "direct virology," to this method of examining viruses from clinical specimens by electron microscopy.

Although second and third generation tests have been developed for the detection of the Norwalk group of viruses and the rotaviruses, the electron microscope is still an indispensable tool for the study of these gastroenteritis viruses. It is also the most rapid diagnostic tool for detection of rotavirus from a clinical specimen and is the only method available for diagnosing infection with certain 27nm viruses associated with epidemic nonbacterial gastroenteritis. It also is important for: (i) providing direct visualization of virus particles from density gradients (to establish their morphologic appearance, e.g., single or double capsid, integrity of capsid structure, and to determine presence or absence of particles or their quantitation); (ii) providing direct visualization of particles from clinical specimens to determine their identity, if feasible; (iii) attempting to visualize the site of activity of antibodies such as monoclonal antibodies or recombinant virus induced antibodies; and (iv) serologic studies performed by immune electron microscopy to determine the antigenic relationships of fastidious gastroenteritis agents that cannot be propagated in cell culture such as the human group C rotaviruses, and the Norwalk group of agents. However, its most important and creative role is in its application to the detection of new, heretofore unknown, agents of acute infectious gastroenteritis and other diseases as well.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00343-09 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

The Role of Norwalk Virus and Related Norwalk-Like Viruses in Viral Gastroenteritis

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	Kim Green, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS (*if any*)

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
<0.1	<0.1	0

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Norwalk virus is one of a group of small, noncultivatable, 27 nm viruses that are important etiologic agents of epidemic gastroenteritis in older children and adults. Attempts were continued this year to cultivate Norwalk virus in tissue culture.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00346-09 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Characterization of the Rotavirus VP4 Gene by Sequencing & Hybridization Techniques

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
Others:	Albert Kapikian, M.D.	Head, Epid. Section	LID, NIAID
	Robert Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS (if any)

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Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previous studies in LID suggested that the low virulence of rotaviruses isolated from neonates undergoing asymptomatic infection in a newborn nursery in which this virus was endemic was determined primarily by the VP4 gene of these strains. This interpretation was based on (i) the high degree of sequence relatedness of the VP4 gene of the M37 strain (originally derived from an asymptomatic newborn baby in Venezuela) with the corresponding gene of other strains recovered in diverse geographic locations from infants who underwent asymptomatic infection in newborn nurseries in which rotavirus was endemic, and (ii) the distinctness of the aforementioned VP4 genes from those present in strains isolated from older infants who had diarrheal disease. To determine whether or not this was a consistent finding, we obtained additional specimens from (i) infants who underwent a silent infection in a nursery in which rotavirus was endemic or (ii) infants who had a sporadic asymptomatic infection in a setting in which rotavirus was not endemic. These specimens collected from diverse geographic locations were examined by a dot hybridization assay or partial sequence analysis of the VP4 gene. Our observations suggest that the M37 VP4 gene allele (now designated as VP4 serotype 2) is associated with persistent transmission of asymptomatic rotavirus infection in newborn nurseries. However, host factors also play a role in sporadic asymptomatic neonatal infection that occurs in nurseries in which rotavirus is not endemic.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00446-06 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Testing of Rotavirus Vaccine Candidates in Venezuela and Peru

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS (if any)

Instituto de Biomedicina, Caracas, Venezuela (Pérez-Schael); Instituto de Investigacion Nutricional, Lima, Peru (Lanata)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	0.6	1.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previous field trials of the rhesus rotavirus (RRV) vaccine in young infants in Venezuela, Rochester, and Arizona have indicated that serotype specific immunity is necessary for protection against rotavirus diarrhea. This goal could be achieved by incorporating into a candidate vaccine (i) a strain which is highly cross-reactive with a wide range of wild type human rotaviruses, or (ii) strains that collectively represent the VP7 serotype repertoire of epidemiologically important rotaviruses. The second approach was investigated in phase 1 studies designed to evaluate the antigenicity and reactogenicity of human rotavirus-RRV reassortants with the VP7 specificity of human serotypes 1 (D x RRV), or 2 (DS1 x RRV). These studies were carried out in Venezuela and Peru. The 2 reassortant vaccines were compared with the candidate RRV vaccine. A quadrivalent vaccine composed of RRV, D x RRV, DS1 x RRV, and a serotype 4 x RRV reassortant (ST3 x RRV) has also been tested in Venezuela. In addition, we have tested a new vaccine candidate developed in LID, the M37 strain, originally isolated from an asymptomatic newborn infant.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00451-04 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunological and Molecular Characterization of Rotavirus Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Albert Z. Kapikian, M.D.	Head, Epid Section	LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visiting Associate	LID, NIAID
	Koki Taniguchi, Ph.D.	Visiting Associate	LID, NIAID
	Kazuo Nishikawa, M.D.	Visiting Associate	LID, NIAID
	Kim Green, Ph.D.	Staff Fellow	LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(if any)*

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Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE 1989-90

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00478-05 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Rotavirus/Adenovirus Recombinants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
Others:	Gisela Larralde	Guest Researcher	LID, NIAID
	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID

COOPERATING UNITS (if any)

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although respiratory transmission of adenovirus types 4 and 7 is associated with respiratory illness, enteric infection with these viruses is clinically silent but results in induction of resistance against respiratory disease due to these strains. Based on this observation, recombinants between these adenovirus serotypes and relevant genes from several viruses (hepatitis B, rabies, measles, HIV, etc.) have been developed. We are applying this approach to develop rotavirus/adenovirus recombinants which are capable of expressing the VP7 rotavirus outer capsid protein and thus could be considered for use as a vaccine candidate.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00507-04 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Characterization of Rotavirus Serotypes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Kim Green, Ph.D	Senior Staff Fellow	LID, NIAID
Others:	Yuan Qian, M.D.	Visiting Associate	LID, NIAID

COOPERATING UNITS (if any)

University of Pavia, Pavia, Italy (Gerna); University of Rochester, Rochester, NY (Madore and Dolin)

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Sequence analysis has become a useful tool for characterization of circulating rotavirus strains. Previous work in our laboratory identified areas of sequence in the gene encoding the rotavirus outer capsid protein VP7 that appear to be associated with VP7 serotype specificity. Thus, strains which fail to react with serotype-specific monoclonal antibodies in an ELISA or which do not grow well in tissue culture can be characterized with molecular techniques. Sequence analysis can also be used to examine antigenic variation at the genetic level. Circulating rotavirus strains from various rotavirus vaccine trials are being analyzed by sequence analysis to determine whether viral antigenic variation is a factor in vaccine failure. In addition, the complete nucleotide sequence of the gene 9 from two "subtypes" of VP7 serotype 4 was determined and compared. It was possible to distinguish subtypes at the nucleotide and amino acid level. Whether subtypes of a serotype are relevant to vaccine development is under investigation.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00532-03 LID

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Expression of Rotavirus Outer Capsid Protein VP4 by a Baculovirus Recombinant

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID
Others:	Gisela Larralde	Guest Researcher	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS (if any)

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Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Rotavirus outer capsid protein VP4 is a major independent protective antigen. A cDNA clone representing the VP4 gene of symptomatic human rotavirus strain KU (VP7 serotype 1) or DS-1 (VP7 serotype 2) or asymptomatic human rotavirus strain 1076 (VP7 serotype 2) was constructed and inserted into a baculovirus expression vector under the control of the polyhedrin promoter. The resulting recombinants expressed the appropriate authentic VP4 protein. Guinea pigs immunized with these VP4 proteins developed specific antibodies which neutralized infectivity of the rotavirus from which the immunizing VP4 was derived. These antisera were then used in neutralization tests to define the extent and distribution of VP4 antigenic polymorphism among human rotaviruses. Three distinct serotypes and one subtype of the VP4 outer capsid protein were identified among 17 human rotavirus strains that had previously been assigned to 5 distinct VP7 serotypes. For the most part, VP4 serotype segregated independently of VP7 serotype.

Among the 10 rotavirus strains whose VP4 gene was previously sequenced, there was complete concordance between assignment of VP4 serotype by neutralization and classification according to VP4 amino acid homology. Thus, rotaviruses that exhibited a VP4 amino acid homology of 89% or greater belonged to the same VP4 serotype as determined by neutralization. Finally, evidence was obtained that the serotype-specific domain is located on the VP8 cleavage subunit of VP4.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00533-03 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Rotavirus Proteins with Monoclonal Antibodies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Kim Green, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS (if any)

University of Tampere, Tampere, Finland (Vesikari, Ruuska)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	0.4	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Monoclonal antibodies have proved to be important reagents for the antigenic analyses of rotavirus proteins. Recently, these antibodies have been used extensively for the identification of VP7 serotype of rotavirus strains recovered from epidemiologic surveys and vaccine trials. Serotype identification of rotavirus field isolates is especially critical for surveys conducted during rotavirus vaccine trials. In addition, several monoclonal antibodies have been adapted in our laboratory for use in an epitope-blocking immunoassay in order to analyze antigenic site-specific antibody responses to infection with wild type rotaviruses or attenuated rotavirus vaccine strains. This technique is being utilized in the analysis of sera from rotavirus vaccine trials so that the immune responses important in vaccine induced protective efficacy can be identified.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00534-03 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Analysis of Non Group A Rotaviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Yuan Qian, M.D.	Visiting Associate	LID, NIAID
Others:	Kim Green, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS (if any)

Ohio State University, Wooster, Ohio (Saif); Ishimaru Pediatric Clinic, Ehime, Japan (Ishimaru); Ehime Prefecture Institute of Public Health, Ehime, Japan (Yamashita)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.9	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although the major goal of the Epidemiology Section is the development of a vaccine against Group (Gp) A rotavirus, it is essential to develop reagents for study of other groups of rotaviruses (B-F) since 2 of these groups, B and C, have also been implicated as etiologic agents of diarrheal disease in humans in Asia. It is not known whether non-group A rotaviruses will emerge as important world-wide pathogens after vaccines for Gp A are used extensively. Strategies for cloning genomic RNA of unknown sequence were developed using the fourth gene of Gp A rotavirus strain 69M as a model. cDNA libraries of a porcine Gp C strain and a human Gp C rotavirus isolate were generated and several partial clones specific for different genomic segments from porcine or human Gp C rotavirus were selected and sequenced. Using this sequence information coupled with the polymerase chain reaction (PCR) amplification technique, it was possible to generate complete gene 8 clones from both human and porcine Gp C rotaviruses and obtain evidence this Gp C gene encodes the outer capsid protein VP7, a major protective antigen. In addition, analysis of the genetic relatedness of porcine and human Gp C rotaviruses indicated that these viruses are closely related genetically and quite distinct from Gp A rotaviruses. However, it appears that the porcine and human Gp C rotaviruses evolved from a common ancestral source.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00558-02 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prediction of Rotavirus Serotypes by Hybridization to Specific Probes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
Others:	Gisela Larralde	Guest Researcher	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to determine the serotype specificity of rotaviruses, an assay based on the use of genetic probes to recognize specific areas of the VP4 and VP7 genes was developed using the polymerase chain reaction (PCR). The assay consists of selectively amplifying (with the use of specific primers) highly divergent regions of the VP4 or VP7 genes associated with neutralization specificity. By incorporating P^{32}-deoxy ATP high specific activity probes were generated and hybridization of the probes to RNAs from rotavirus positive stool suspensions or infected tissue culture lysates was used to identify serotype.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00559-02 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Characterization of Gene Segment 1 of the Gottfried Porcine Rotavirus Strain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Norio Fukuhara, Ph.D.	Visiting Fellow	LID, NIAID
Others:	Kazuo Nishikawa, M.D.	Visiting Associate	LID, NIAID
	Mario Gorziglia, Ph.D.	Visiting Associate	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1989-90

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00560-02 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The VP6 Gene of Equine Rotavirus Strain H-2 Encodes Two VP6 Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mario Gorziglia, Ph.D. Visiting Associate LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1989-90

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00561-02 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Genomic Rearrangement in Rotaviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Mario Gorziglia, Ph.D.	Visiting Associate	LID, NIAID
Others:	A.Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1989-90

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00573-01 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Expression of Rotavirus Proteins in *Salmonella* Bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kim Green, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Yuan Qian, M.D.	Visiting Associate	LID, NIAID
	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(if any)*

University of Maryland, Baltimore, Maryland (Levine, Hone, Losonsky)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was initiated in our laboratory with the goal of developing a recombinant rotavirus vaccine using attenuated *Salmonella* strains as the expression system. In this project, it is hoped that key antigens involved in the development of broadly-protective immunity to rotavirus will be identified. *Salmonella* offers many advantages as an expression system, the foremost being the delivery of antigens to the mucosal surface of the gut. Thus, mechanisms associated with rotavirus immunity can be studied with specific antigens of the virus at the site where the virus adsorbs and replicates. This information is relevant to the improvement of current vaccine strategies and to the development of future recombinant vaccines.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00574-01 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of Rotavirus Outer Capsid VP4 Subunits (VP8 and VP5) in *E. coli*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Gisela Larralde, M.S.	Guest Researcher	LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The VP5 and VP8 cleavage subunits of the VP4 outer capsid protein of human rotavirus strain KU (VP7 serotype 1), DS1 (VP7 serotype 2) or asymptomatic human rotavirus strain 1076 (VP7 serotype 2) were expressed in *E. coli*. Immunization of guinea pigs with the VP5 or VP8 protein of each strain induced antibodies that neutralized the rotavirus from which the VP4 subunits were derived. The results obtained by cross-immunoprecipitation and reciprocal neutralization assay using antisera to the VP5 and VP8 expressed proteins suggest that the VP8 subunit of VP4 contains the major antigenic site(s) responsible for serotype-specific neutralization of rotavirus via VP4, whereas the VP5 subunit of VP4 is responsible for the cross-reactivity observed among strains that belong to different VP4 serotypes.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00575-01 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of the Outer Capsid Protein of Human Rotaviruses in Infectivity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Gisela Larralde, M.S.	Guest Researcher	LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The binding of the VP4 protein to MA104 cells, a permissive host for rotavirus infection, was demonstrated using lysates of insect *Spodoptera frugiperda* (Sf9) cells that had been infected with a recombinant baculovirus expressing the VP4 gene of human rotavirus strain KU, DS-1, or 1076. Moreover, binding assays using the *in vitro* expressed VP5 or VP8 subunit of VP4, indicated that VP5 and not VP8 was responsible for the binding of the VP4 protein to a susceptible host cell.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00576-01 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Serotype Analysis and Characterization of Rotaviruses from Malaysia

PRINCIPAL INVESTIGATOR (List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Nassar Rasool, Ph.D.	Guest Researcher	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID
	Kim Y. Green, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A total of 341 stool specimens known to be positive for group A rotaviruses by RNA electrophoresis had been obtained from various locations in Malaysia from children below the age of five years who had gastroenteritis. These stool specimens which were collected from 7 of the 12 years spanning 1977-1988 were brought to the NIH for determination of the serotypes circulating in Malaysia and for further characterization by molecular biologic techniques. Ten percent suspensions of 294 specimens were tested for antigen by a pre-post confirmatory ELISA. Forty-seven specimens appeared to be too dilute or were limited in quantity and thus, were not tested by this system, but rather were passaged into primary African green monkey kidney (AGMK) cells using the method of Ward *et al.* (1984). Of the 285 specimens that were ELISA positive, 176 could be serotyped: 71% belonged to serotype 4, 15% to serotype 1, 4% to serotype 3 and 3% to serotype 2. In addition, 6% displayed ambiguous serotype specificities, reacting to more than one monoclonal antibody, and need to be investigated further.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00577-01 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Characterization of the VP4 Gene of Strain 69M, a New Human Rotavirus Serotype

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Yuan Qian, M.D.	Visiting Associate	LID, NIAID
Others:	Kim Green, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human group A rotavirus, strain 69M, isolated from an ill child in Indonesia, has been described as a new serotype (serotype 8). Previous sequence analyses in our laboratory of the gene encoding the outer capsid protein VP7 confirmed that 69M is distinct from established human rotavirus serotypes 1, 2, 3 and 4. In order to assess whether, VP4 (encoded by gene 4), the other major outer capsid neutralization protein of 69M is also distinct, the complete nucleotide sequence of the gene 4 was determined. The sequence of the 69M VP4 gene was compared with available VP4 gene sequences from human and animal rotaviruses. In addition, Northern blot hybridization was performed to examine the genetic relatedness of the 69M VP4 gene with other group A rotaviruses using ^{32}P-labeled cloned 69M VP4 gene DNA or 69M ssRNA transcript probes. These genetic studies as well as neutralization confirmed that the fourth gene (encoding VP4) of strain 69M is different from any of the other corresponding genes described thus far. Of interest, comparative analyses of the deduced amino acid sequences suggest that the VP4 of 69M is more closely related to animal rotaviruses than to human rotaviruses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00582-01 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression and Recovery of Recombinant Rotavirus VP4 from Insect Cell Cultures

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Mario Gorziglia, Ph.D.	Visiting Associate	LID, NIAID
Others:	Luis G. Juarbe-Osorio	Guest Researcher	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
<0.1	<0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The possibility of producing and purifying large quantities of recombinant rotavirus VP4 from insect cell cultures was investigated. *Spodoptera frugiperda* (Sf9) cells were grown in large spinner flasks and infected with a baculovirus expressing the rotavirus OSU VP4 gene. The expressed VP4 subsequently was purified from such cultures to determine the yield of recombinant protein. Conditions for growth of cells and high yield of VP4 were optimized to improve the expression of VP4. Also, a practical purification scheme was developed to maximize the yield of VP4 from insect cell cultures and to reduce cost and complexity of a potential industrial-scale purification process.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00583-01 LID

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of the Outer Capsid Protein VP4 of Human and Porcine Rotaviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Baoguang Li, Ph.D.	Visiting Fellow	LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The full-length of cDNA of the VP4 of the human rotavirus K8 strain and the porcine rotavirus Gottfried strain were cloned into the pGEMEX system and expressed in *E. coli* (JM 109) by induction with IPTG. The VP4 outer capsid protein of each of these strains was expressed at a high level in *E. coli*. In addition, both the VP5 and VP8 subunits of VP4 derived from the K8 and Gottfried strains were expressed in *E. coli*. The yields of the expressed VP5 and VP8 subunits were much higher than that of expressed VP4. Expression of the full-length of VP4, VP5, and VP8 in *E. coli* provides an efficient source for these proteins and should facilitate research on molecular characterization of the VP4 outer capsid protein.

PHS 6040 (Rev. 1/84) GPO 914-918

LABORATORY OF MOLECULAR MICROBIOLOGY
1990 Annual Reports

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LABORATORY OF MOLECULAR MICROBIOLOGY

SUMMARY

The Laboratory of Molecular Microbiology (LMM) applies molecular and biological techniques to study the structure, function, and regulation of prokaryotic and eukaryotic genes. Programmatically, a wide range (murine retroviruses, the human immunodeficiency virus (HIV), HTLVI, SIV, yeast, and mycoplasmas) of microorganisms and their host cells are investigated. Members of the LMM are physically located in Building 4 on the Bethesda campus and in Building 550 at the Frederick Cancer Research Facility. Although the research activities conducted by the LMM staff are quite diverse, a common theme involves the characterization of genetic elements (both chromosomal and episomal) affecting the interaction of microorganisms and the cells they infect.

STUDIES OF THE HUMAN IMMUNODEFICIENCY VIRUS

Molecular biololgical and virological techniques have been employed to dissect structure/function relationships of structural and regulatory genes of HIV during acute and chronic virus infections. The approach followed has tended to focus on the effects of mutated viral genes evaluated in the context of the complete viral genome; complementary <u>in vitro</u> analyses of isolated HIV genes have also been carried out.

<u>Structural and functional analysis of the HIV vif protein</u>. Mutation of the HIV <u>vif</u> gene has previous been shown to reduce virus infectivity 100 to 1000 fold. The 23 kDa <u>vif</u> protein is present at relatively low concentrations inside infected cells. The protein is very insoluble, is not membrane-associated, and is not a component of virus particles. <u>Vif</u> apparently exists as a monomer and is not modified, post-translationally. Its absence from virions coupled with low intracellular concentrations suggests an enzymatic rather than a structural function. Biochemical analyses indicate that <u>vif</u> does not affect the expression or processing of other HIV proteins or their assembly into progeny particles. <u>Vif</u>-minus virus mutants contain normal amounts of genomic RNA and reverse transcriptase and are capable of synthesizing cDNA <u>in vitro</u>.

(Strebel and Martin).

<u>Structural and functional analysis of the HIV vpu gene</u>. Our previously published work indicated that the HIV <u>vpu</u> protein mediates the release of progeny virions from virus-producing cells. Although high concentrations of <u>vpu</u> are present intracellularly, none can been detected associated with particles. <u>Vpu</u> is an integral membrane protein and is predominently located in the perinuclear regions of virus infected cells. <u>In vitro</u> analyses revealed that the hydrophilic C-terminus of <u>vpu</u> extends to the cytoplasmic side of the plasma membrane. <u>Vpu</u> is phosphorylated and forms homopolymeric complexes, most likely tetramers, in virus producing cells.

(Strebel and Martin)

Identification of cis-acting inhibitory sequences associated with the HIV genome. A novel recombinant plasmid containing the HIV tat gene was constructed and an assay developed to identify and characterize "negative"-acting sequences in the HIV genome. Segments of the HIV provirus encompassing gag/pol sequences were cloned downstream of tat and their inhibitory effect on tat expression was monitored following transfection of an HIV LTR-directed chloramphenicol acetyl transferase containing human cell line. Two independent elements were identified (one mapping in the gag and the other in the pol gene) in the HIV genome. The inhibitory effect of these elements was verified in a heterologous test system and shown to be orientation dependent.

(Maldarelli and Strebel)

The role of NF-κB /Sp1 elements in HIV-1 replication. An infectious molecular clone of the HIV provirus, with a mutagenized long terminal repeat (LTR) containing no NF-kB or Sp1 sites, failed to replicate in any human cell examined. NF-kB and/or Sp1 elements were added singly or in combination to this defective HIV LTR which was then reintroduced into a full length cloned proviral DNA and virus stocks prepared following transfection. The minimal enhancer element proved to be juxtaposed NF-kB and Sp1 segments which are present in all primate lentiviruses. Virus derived from such constructs replicated efficiently in all human T-cells tested. In some cells, virus stocks containing a single NF-κB and no Sp1 sites replicated but exhibited delayed infection kinetics.

(Ross, Rabson and Martin)

Characterization of HIV U5 LTR mutations and their effect on viral replication. The U5 region of HIV LTR was mutagenized and analyzed in the context of an infectious proviral DNA. The deletion of 26 nucleotides in the center or 3' one-third of the U5 portion of the HIV LTR generated infectious virions which exhibited delayed kinetics of replication compared to the wild type parent. In contrast, the deletion of 29 nucleotides, located in the 5' one-third of U5 completely abolished infectivity. These findings suggest that the 5' end of the U5 region of HIV LTR contains elements critical for viral replication, whereas the middle portion and the 3' one-third contribute to efficient viral replication.

(Vicenzi and Martin)

Characterization of the U3 region of HIV LTR upstream of the promoter/enhancer elements. The 5' end of the HIV U3 segment has been reported to bind to several transcription factors. In order to study the functional role of this region in virus replication, deletion mutations were introduced in the context of an infectious proviral DNA. Since such alterations would interrupt the *nef* open reading frame, all the mutations were analyzed in a *nef* minus mutant. Interestingly, the *nef* minus mutant virus containing a wild type U3 region, replicated very poorly even though more extensive cell killing, compared to the wild type virus infection, occurred. The U3 LTR mutations restored the levels of viral replication and reduced the cytopathicity induced by the *nef* minus virus to wild type levels.

(Vicenzi and Martin)

Role of the LTR Sp1 binding sites in HIV replication. The function of the HIV LTR Sp1 binding sites during virus infection was examined by constructing site-specific mutations in which all three Sp1 sites were deleted from the LTR. HIV deleted in these sites continued to replicate in human T cells containing NF-κB binding activity (MT4 cells and PHA-

stimulated peripheral blood lymphocytes) but not in A3.01 cells, a human T-cell line which lacks detectable NF-κB activity. If NF-κB activity is induced in A3.01 cells by treatment with tumor necrosis factor-α, these cells became permissive for replication of the Sp1 deleted mutant of HIV. These results suggest that NF-κB can functionally substitute for Sp1 in the regulation of HIV gene expression and are consistent with a model in which these two sites function as "enhansons" in activating HIV gene expression. Recently, a "second site" revertant of the Sp1 deleted virus has been isolated which is capable of growing in A3.01 cells despite the absence of any Sp1 binding sites; the revertant virus contains a duplication of the 3' NF-κB binding site. The apparent ability of HIV to alter its host range through mutations in promoter regulatory elements may have implications for the pathogenesis and progression of HIV infection.

(Rabson)

Role of the NF-κB elements present in the HIV-1 LTR in viral replication and cytopathicity. Several infectious molecular clones of an HIV-1 isolate (AL1) were characterized and found to contain natural deletions or duplications affecting the two NF-κB binding domains present in all reported HIV genomes. A deletion involving 13 base pairs including the first five nucleotides of the 5'-most NF-κB element delays virus replication in MT-4 cells by 2 days. The same deletion, in combination with other mutations in the viral genome, confer a phenotype of slow growth and reduced cytopathicity, resulting in a chronic and only mildly cytocidal infection which can be sustained indefinitely. In CAT assays, the enhancer activity of LTRs containing the NF-κB deletion is significantly less than that of an LTR containing either two or three intact NF-κB elements. The triplicated NF-κB configuration is highly unstable during virus passage, giving rise to deletions of the 5'-most element and/or the central element.

(Englund, Martin and Hoggan)

Characterization of the HIV-1 LTR target for Tat. *Tat* activates HIV-1 LTR directed gene activity via the TAR element in nascent RNA as well as through DNA elements within U3. Deletion of the NF-κB and Sp1 motifs from U3 renders the LTR nonresponsive to *tat.* Similarly, the deletion of critical TAR sequences or the disruption of TAR secondary structure in nascent viral RNA by *in vitro* mutagenesis severely impairs *tat* trans-activation.

(Berkhout and Jeang)

A cellular protein binds to TAR RNA. Using a lambda gt11 cDNA library, a human cellular sequence was isolated which encodes a protein that binds to HIV-1 TAR RNA in a functionally specific fashion. This cDNA, when driven by the strong constitutive CMV promoter, is able to trans-activate many eukaryotic pol II promoters including the HIV-1 LTR. The sequence for this cDNA has been completely determined and does not correspond to known cellular transcriptional factors.

(Gatignol and Jeang)

Mechanism of HIV-1 gene regulation by the *rev* protein. The replication of HIV-1 requires the function of a 21 kDa nuclear protein, *rev*. *Rev* interacts with a highly structured viral mRNA sequence, RRE (*rev* responsive element) a 236 bp segment located in the *env* gene thereby facilitating the nuclear export of viral mRNAs containing RRE. The critical functional elements of RRE fold into a structure composed of a stem-loop A, formed by the

ends of the RRE, joined to a branched stem-loop B/B1/B2, between bases 49-113. Maintenance of the native RRE secondary structure alone was not sufficient for *rev* recognition. Mutations that changed a 5'..CACUAUGGG..3' sequence in the B stem, without affecting the structure abolished the *rev* response. Large quantities (2-3 mg/10^9 cells) of biochemically active *rev* was also expressed using a TK- vaccinia virus recombinant.

(Holland, Ahmad, Maitra, Wingfield and Venkatesan)

Characterization of vif mutants of HIV-1 and HIV-2. The vif protein of HIV-1 has been reported to be necessary for cell-free transmission of virus, since vif mutants can only be passaged by co-culture of transfected cells, presumably by a cell-to-cell transfer mechanism. To confirm and extend these findings, mutations have been introduced into the vif genes of the BRU isolate of HIV-1 and the ROD isolate of HIV-2 and both the *vif* and *vpr* genes of BRU. The vif mutant of HIV-2 could not be propagated by either cell-free infection or co-cultivation. For the BRU isolate of HIV-1, the phenotype observed depended on the cells used for the infection. For example, the vif mutation and the *vif-vpr* double mutation in BRU resulted in a delay of about two days in progeny virus production CEM and U937. In contrast, a delay of four to five days was observed in SupT1 cells, and in H9 cells, no progeny virus production was detected.

(Peden)

Analysis of infectious clones of two HIV-1 isolates from Zaire. The assembly of proviral molecular clones of two HIV-1 isolates from central Africa, MAL and ELI, started at the Pasteur Institute, was recently completed. In contrast to the results with the HIV-1 BRU isolate, not all cell lines tested were permissive for virus prepared from the MAL and ELI infectious clones. MAL could replicate on H9 and SupT1 cells, but no viral growth was detected on CEM and U937 cells. In contrast, ELI could infect CEM and U937 cells but not H9 nor SupT1 cells. The mechanisms underlying these differences in cell tropism is being investigated by exchanging homologous segments of the ELI and MAL cloned proviruses.

(Peden)

HIV-1 Envelope Processing. The intracellular transport and processing of the HIV-1 envelope proteins have been studied using wild-type and envelope mutant proviral clones. Pulse-chase experiments in conjunction with several biochemical assays have been conducted on lysates prepared from acutely infected cells or following transfection of wild type or mutant proviral clones. The cumulative results from these analyses indicate that intracellular transport and processing of the gp160 precursor polyprotein proceeds via the rough endoplasmic reticulum (ER) and Golgi complex. The formation of oligomeric gp160 structures within the ER may be required for transport; however, appropriate folding after oligomerization is also a critical step. Mutations within different regions of the envelope can alter the intracellular distribution and fate of the gp160 as well as the envelope cleavage products, gp120 and gp41. Intracellular sorting of the HIV-1 envelope glycoproteins appears to very complex and may ultimately determine which proteins become incorporated into viral particles.

(Willey and Martin)

HIV-1 envelope structure and function. Oligonucleotide-directed site-specific mutagenesis has been used to introduce amino acid changes into two discrete regions of the HIV-1 envelope gene. These regions have been previously reported to be critical for fusing viral and cellular membranes. In addition to affecting the fusion reaction, all of the mutations impair or ablate the replicative capability of HIV-1. Quantitative analyses of envelope processing revealed that some mutations dramatically reduce the intracellular production of the mature envelope components gp120 and gp41 while others affect the stability of these proteins after processing. These results indicate that individual regions within the viral envelope have multiple roles (*viz.* envelope structure, function, and processing).

(Willey and Martin)

Monotropic HIV-1 isolates replicate efficiently in CSF-1 treated, primary monocyte-derived macrophage cultures. We have developed a primary monocyte-derived macrophage tissue culture system in which the growth of the monotropic AD-87 isolate of HIV-1 replicates with an efficiency comparable to other HIV isolates in T-cells. Pre-treatment of the cells in suspension with recombinant human CSF-1 for forty-eight hours, followed by plating at a density of at least 10^6/well in 24-well plates, gives rise, 9 days later, to a culture competent to replicate the AD-87 isolate with high efficiency. Using this system, in combination with an "inside-out" PCR method developed in our laboratory, we have demonstrated that the AD-87 proviral DNA becomes integrated into the genome of infected monocyte-derived macrophages.

(Englund and Martin)

Molecular cloning and biological characterization of a monotropic isolate of HIV-1. Infectious molecular clones of the AD-87 isolate were derived from unintegrated proviral DNA in a primary lymphocyte infection. All of these clones direct the synthesis of HIVs which replicate in primary lymphocytes. One of the clones also generates virus which efficiently infects primary, monocyte-derived macrophages. These results indicate that: 1) The AD-87 isolate is a mixed population of HIV-1, containing both T-cell restricted and broader host-cell range variants, and 2) the broad host-cell range phenotype is a property of an individual molecular clone.

(Englund and Martin)

UV irradiation increases HIV LTR driven expression of cholaramphenicol acetyl transferase (CAT) in HIV-LTR/CAT transgenic mice. UV irradiation is known to induce HIV-directed gene expression in tissue culture systems. To determine whether UV light induces HIV gene expression *in vivo*, transgenic mice harboring HIV-LTR driven CAT gene were exposed to UV radiation. Increased CAT expression (up to 30-fold) was observed in ear samples from irradiated animals. The kinetics of *in vivo* UV induced CAT activity reached a maximum on day 3 and diminished to baseline levels by day 7. These data suggest that UV light can also increase HIV LTR driven expression *in vivo* and may prove useful in activation of latent proviruses in animal models.

(Frucht, Vicenzi and Martin)

Biochemical characterization of the HIV *nef* protein. HIV *nef* protein has been reported to share certain biochemical and structural properties with oncogenes of the *ras* family. To determine whether this is a general property of *nef* from various HIV isolates, *ras* and *nef*

clones, expressed in the same bacterial and mammalian vectors, were compared biochemically and biologically. Unlike *ras, nef* proteins lacked GTP binding activity but exhibited autophosphorylation with either GTP or ATP as the phosphate donor. In addition, unlike *ras*, HIV *nef* did not exhibit oncogenic potential in focus-forming assays with NIH 3T3 cells nor caused meiotic maturation of *Xenopus* oocytes. It therefore appears that the biological function of *nef* does not follow the pattern of G proteins. However, the *nef* associated phosphorylation, either by protein kinase C or through its autokinase activity, could be functionally significant *in vivo.*

(Nebreda, Bryan, Segade, Wingfield, Venkatesan, Santos)

OTHER RETROVIRUSES

HTLV-I Tax and Rex proteins have multiple functional activities. Over 50 point mutations were inserted in the *tax* gene of HTLV-I and have been used to define functional domains within the *tax* protein. Experiments correlating structural changes with *tax* function are presently in progress. The *rex* protein has been found to induce increased transcriptional initiations from a variety of eucaryotic promoters. This activity differs from the previously described post-transcriptional function for *rex* which modulated the nuclear to cytoplasmic transport of viral mRNA.

(Semmes, Nelson and Jeang)

Mouse Leukemia Virus Cell Surface Receptors. The mouse leukemia viruses comprise four different subgroups defined by interference patterns and host range. A mouse cDNA that confers susceptibility to the ecotropic host range group was used to map this gene to chromosome 5 confirming that it is likely to be *Rec-1*, the ecotropic cell surface receptor. This gene was also positioned at the distal end of chromosome 5 by analysis of an interspecies backcross demonstrating that *Rec-1* is not part of the cluster of retrovirus-associated genes near the centromere, and also demonstrating that *Rec-1* does not map at or near any known mouse mutation that might suggest its normal role. A second gene, *Tea*, cloned by C. MacLeod by virtue of its differential expression in T cells, was found to be highly homologous to *Rec-1*. This homology suggests that *Tea* and *Rec-1* define a new family of membrane spanning proteins and suggests that *Tea* may represent one of the other MuLV receptor genes. Since *Tea* maps to chromosome 8 it is distinct from *Rec-1*. However, *Ram-1*, the gene encoding the receptor for amphotropic MuLVs maps to this same chromosome suggesting that *Tea* may encode this MuLV receptor.

(Kozak)

A highly divergent SIV from Asian stump-tailed macaques. A 2.0 kb DNA fragment containing the *gag* region of SIV_{stm} has been amplified by PCR from virus-infected human peripheral blood lymphocyte DNA. Nucleotide sequence analysis of SIV_{stm} *gag* segment indicated that it shared 84% homology with the *gag* genes of SIVs isolated from Asian pig-tailed (SIV_{mne}) and cynomolgous (SIV_{mac}) macaques and African sooty mangabey (SIV_{smm}). SIVmme and SIVmac (which are 96% homologous to each other and 87% with

SIV_{smm}) are thought to have originated in captivity by cross-infection of Asian macaques with SIV from the African sooty mangabey. The nucleotide sequence divergence of SIV_{stm} from the other SIVs suggests that the stump-tailed macaque was infected by a virus different from that of the sooty mangabey.

(Galvin and Khan)

Evaluation of RNase H activity in tissue culture systems. Mutations of conserved amino acids in the C-terminal portion of the Moloney murine leukemia virus (M-MuLV) and HIV RNase H coding sequences suppressed RNase H activity *in vitro* ore than 100-fold with little effect on reverse transcriptase activity; host cells transfected with mutant proviruses produced noninfectious particles. This presumed RNase H defect was confirmed *in vivo* (M-MuLV) by PCR amplification of minus strand DNA produced in cells transfected with mutagenized proviral DNA. Transcription of minus strand strong stop DNA was observed but full-length minus strand DNA synthesis did not occur.

(Repaske)

A highly sensitive and rapid assay for HIV reverse transcriptase has been developed. The HIV RT assay performed on infected tissue culture supernatant fluids has been used as an indicator of virus replication. This assay, as currently conducted, lacks the sensitivity of the p24 ELISA assay and is not directly proportional to virus concentration. The requirements for optimal RT activity have been analyzed in detail and used for the development of an assay that responds directly to virus concentration and time; RT determinations are now almost as sensitive as p24.

(Repaske)

ONCOGENESIS

Oncogenes involved in MuLV-induced neoplastic Disease. MuLVs are capable of transforming mouse cells by mechanisms which include transduction of cellular oncogenes and activation of oncogenes by insertional mutagenesis. The cellular loci corresponding to a new virally transduced oncogene has been characterized and an unusually modified Ha-*ras* oncogene associated with a T cell tumor has been identified. The acute transforming virus, CasNS-1, was derived from a replication competent virus isolated from mice from Lake Casitas, California. This virus causes pre-B cell neoplastic diseases, and was found to carry novel retroviral as well as cellular sequences. The cellular sequences were cloned and shown to be represented in the mouse genome in two copies (*Cbl-1* and *Cbl-2*). One copy lacked introns and is assumed to be a processed pseudogene; the other contains introns and maps to a chromosomal region which has been implicated in many neoplastic diseases. In the second study, experiments were done to characterize the cellular sequences flanking a retroviral integration in a T cell leukemia. These cells were found to express high levels of Harvey *ras*; the *Hras-1* gene had been disrupted by the insertion of retroviral sequences as well as by an intrachromosomal rearrangement.

(Kozak)

Glycerophosphoinositol and oncogenic transformation. Transformation of rodent fibroblasts by cytoplasmic (*mos,raf*) and membrane-associated (*ras,src,met,trk*), but not nuclear (*myc,fos*) oncogenes, results in a consistent and significant elevation of intracellular glycerophosphoinositol (GPI) levels. Our findings indicate that these high levels of GPI result from deacylation of lysophosphatidylinositol released by intracellular PLA2 activity enhanced at a posstranscriptional level in transformed cells. GPI is a water-soluble, easily detectable metabolite which may constitute a convenient biochemical marker for malignant transformation by this particular group of oncogenes.

(Alonso, Bryan and Santos)

Ras and the insulin pathway. Previous evidence suggested that *ras* proteins participate in the process of meiotic maturation induced by insulin in amphibian oocytes. To check whether *ras* proteins also participate in the intracellular insulin signal transduction pathway, the mammalian cell line NIH3T3 L1, which is known to undergo adipocytic differentiation in a process triggered specifically by insulin or IGF, was examined. In this system, transfected *ras* oncogenes could substitute for insulin in inducing the differentiation process. This effect was specific for *ras* oncogenes since other tyrosine kinase oncogenes, (e.g. *trk*) failed to trigger differentiation.

(Benito and Santos)

The role of *ras* on germinal vesicle breakdown. Treatment of *Xenopus* oocytes with progesterone or insulin or microinjection of transforming *ras* proteins (p21-Lys12) results in germinal vesicle breakdown (GVBD) which is associated with a specific pattern of phosphorylation. This same pattern of phosphorylation was observed after microinjection of normal *ras* protein (p21-Gly12), although the kinetics were significantly slower. Treatment with cycloheximide completely blocked meiotic maturation and associated phosphorylation induced by progesterone and insulin but not by the normal or transforming p21 *ras* proteins. These observations suggest that *ras* proteins may act directly on the cascade of phosphorylation events leading to GVBD.

(Nebreda, Bryan, Santos)

GENE MAPPING

Genetic Linkage Studies in the Mouse. The production of a high density mouse linkage map is an important first step in the characterization of mammalian genomes. A variety of mouse genes have been mapped to specific positions within mouse chromosomes by Southern blot analysis of DNAs prepared from a panel of Chinese hamster X mouse somatic cell hybrids and from the progeny of an interspecies backcross. The somatic cell hybrids have been used to map hundreds of genes to date, and the progeny of this specific backcross have now been typed for over 150 loci mapped to all 20 linkage groups. Some of the recently mapped genes include these responsible for inherited disorders such as retinal degeneration (*rd*), enzymes (*Gba*), and zona pellucida proteins (*Zp-2*). This interspecies

backcross has also been used to describe deviations from the 1:1 segregation ratio expected for individual loci, and has suggested that this type of distortion is not restricted to specific species combinations.

(Kozak and Chakroborti)

Identification of highly polymorphic sequences in man useful for gene mapping. Previous studies from other laboratories have shown that tandem repeats of short sequence motifs such as $(CA)_n$ ("micro-satellites") are highly polymorphic for the number of repeats at a given locus in different individuals. We noted that the 3' ends of many published *alu* sequences contain micro-satellite-like sequences. To see if these sequences were polymorphic, PCR was used to amplify segments of genomic DNa from several genes which contain *alu* sequences. The results obtained indicated that for 2 of 8 loci examined, highly polymorphic micro-satellites were found at the 3' ends of internal *alu* sequences. Since there are 10^5 to 10^6 copies of the *alu* repeat per haploid genome, on average one copy every few kb, our results point to an extremely rich source of readily identified, highly polymorphic sequences which could be used to create a dense genetic map, a primary goal of the Human Genomic Initiative.

(Silver and Nahor)

MYCOPLASMAS

Biological characterization of AIDS-derived mycoplasmas. Three mycoplasmas, which were either associated with AIDS and non-AIDS infections or cultivated directly from blood cells of HIV positive or AIDS patients, were characterized. Strain Mi (Lo agent) was shown to be serologically related to *Mycoplasma fermentans*, and subsequent genetic characterization (guanine + cytosine base composition, DNA restriction endonuclease digestion patterns, and DNA/DNA hybridization) confirmed this identification. Strain 8YH (Montagnier), cultivated in SP-4 medium directly from leukocytes of an asymptomatic HIV sero-positive individual, was also identified as *M. fermentans* by serologic testing. Strain Ber (Montagnier), cultivated from leukocytes of AIDS patient, was shown to be a strain of *M. pirum*, a species heretofore only identified in cell cultures. Documentation that *M. pirum*, is of human origin might offer retrospective insight into the frequent occurrence of this organism in cell cultures established from primary human tumor cell material.

(Tully, Rose, Bove, Lo, and Montagnier)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00027-23-LMM

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Basic Studies of Mycoplasmas

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. G. Tully	Head, Mycoplasma Section	LMM, NIAID
Others:	David L. Rose	Research Microbiologist	LMM, NIAID

COOPERATING UNITS (if any)

J.B. Baseman, Univ. Texas, San Antonio, TX; J.M. Bove, Univ. Bordeaux, France; S.C. Lo, AFIP, Washington, DC; L. Montagnier, Pasteur Inst. Paris.

LAB/BRANCH

Laboratory of Molecular Microbiology

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Mycoplasma Section

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2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mycoplasmas associated with or isolated from tissues of AIDS and non-AIDS patients have been characterized serologically and compared to reference mycoplasmas of human origin maintained in this laboratory. In collaborative studies using both serologic and genetic markers, we were able to provide specific identification of the organisms as to *Mycoplasma* species. Several strains of *M. fermentans* were identified. The new *M. fermentans* strains isolated from humans or from cell cultures were genetically and serologically homogenous to the type strain (PG18) isolated over 30 years ago from the human urogenital tract. At least two isolates of *M. pirum* were identified through serologic and biochemical markers. Both strains had been cultivated initially by a collaborating laboratory from primary leukocyte cultures of AIDS patients, employing SP-4 mycoplasma culture medium developed earlier in this laboratory. This was a significant observation, since all previous isolations of this species had been from cell cultures and there existed no well documented information on the possible host origin of this mycoplasma.
M. genitalium was also identified by the collaborating laboratory in a washed erythrocyte suspension from a patient with AIDS-related complex. Although attempts to culture the organism from the primary erythrocyte material was unsuccessful, DNA implication tests using primers specific for the organism (and capable of differentiating *M. pneumoniae*) were positive for *M. genitalium*. Current attempts are underway to cultivate the organism from this and other AIDS-derived clinical material.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AI 00190-12 LMM

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Molecular Genetics of Eukaryotic Cells and Their Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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	Elizabeth, Ross	Biololgist	LMM, NIAID
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6	3	3

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

UV irradiation is known to induce HIV LTR-directed gene expresssion in tissue culture systems. Transgenic mice, harboring constructs consisting of the HIV LTR linked to the chloramphenicol acetyl transferase (CAT) gene, were exposed to UV-B or UV-C light sources. Increased CAT expression was detected in ear specimens from irradiated transgenic animals. CAT activity reached a maximum during the 7 hr following irradiation and decreased to basal levels by day 7.

In vitro mutagenesis techniques have been employed for investigations of the role of *cis*-acting HIV LTR elements during productive virus infection. We previously ascertained that removal of both the NF-κB and Sp-1 motifs abolished virus infectivity. In a series of "reconstruction" experiments, NF-κB and Sp-1 elements, either singly or in combination, were added to an "empty" LTR associated with an infectious molecular clone of HIV. Virus stocks were obtained by transfection and tested for infectivity and cytopathicity. Our findings indicate that juxtaposition and tandemization of both elements critically influence HIV promoter strength. Mutagenesis affecting the U5 region of the HIV LTR was also carried out. The deletion of 26 nucleotides in the center or 3' one-third of U5 resulted in virus stocks with only modest reductions in replicative capacities. In contrast, deletion of 29 nucleotides positioned in the 5' third of U5 completely abolished replication. These results suggest that the 5' portion of the HIV U5 region contains heretofore unrecognized sequences/elements that are critical for infectivity; the results of preliminary experiments suggest that they may participate in RNA termination/polyadenylation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00218-09-LMM

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical and Chemical Studies on Retroviral DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Roy Repaske	Research Chemist	LMM, NIAID
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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Infectious Moloney murine leukemia virus (M-MuLV) and HIV are non-infectious when the genome is modified by either of two point mutations that change codons for two conserved amino acids in the C-terminal region of reverse transcriptase (RT). We have demonstrated in partially-purified cloned RTs from each of these viruses that polymerase is essentially as active as wild-type RT; however, the RNase H function is severely restricted. Defective M-MuLV particles produced by transformed NIH 3T3 cells were used to infect normal 3T3 cells to determine the kinetics of minus strand DNA synthesis by PCR analysis. Infectious and mutant virus produced (-) strong stop DNA and the "jump" within 15 min after infection, but minus strand DNA did not extend beyond U3. These results confirm in an *in vivo* system that polymerase function in the mutant virus is adequate, whereas inadequate RNase H function is probably the cause of the defect.

The HIV RT assay performed on infected culture supernatant fluids is used as an indicator for the presence of HIV. This assay is not directly proportional to virus concentration and it lacks the sensitivity of the p24 ELISA assay. We have analyzed the requirements for RT activity and have developed an assay that now responds proportionally to enzyme concentration, and the assay is almost as sensitive as p24 detection (a factor of 2 - 3).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00300-09-LMM

PERIOD COVERED October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Genetic Aspects of Viral Oncogenesis in Wild Mouse Species and Laboratory Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C. A. Kozak	Res. Microbiologist	LMM, NIAID
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3	2	1

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Genetic studies on virus-induced neoplastic disease have led to the identification of many chromosomal genes involved in these processes. Many mouse genes affecting susceptibility to virus or virus-induced disease have been identified in the various inbred laboratory strains, and we have expanded these studies to include wild mouse populations because of their greater genetic diversity. We have identified and characterized a novel wild mouse virus, HoMuLV, isolated from Eastern European mice. This virus is pathogenic, has ecotropic host range despite its different envelope gene, and also differs from other murine leukemic viruses in its *gag* gene. In other experiments, we characterized the novel oncogenic sequence transduced by a defective pathogenic wild mouse virus. Two copies of this oncogene were identified in the mouse genome, one of which is a pseudogene. We also demonstrated that activation of the Harvey *ras* oncogene was associated with the development of a T cell leukemia and that this activation was accomplished by viral integration and chromosomal rearrangement. Finally, we positioned the gene encoding the ecotropic MuLV cell surface receptor and characterized a related genomic sequence which may represent the amphotropic MuLV receptor gene.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00301-09-LMM

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Genetic Mapping of Mouse Chromosomal Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C.A. Kozak	Res. Microbiologist	LMM, NIAID
Others:	R.R. O'Neill	Staff Fellow	LMM, NIAID
	B. Mock	Sr. Staff Fellow	NCI
	J. Dean	Section Chief	NIDDK
	S. Kimura	Guest Worker	NCI
	F. Gonzalez	Section Chief	NCI
	D. Goldman	Section Chief	LCS, NIAA

COOPERATING UNITS (if any)
H. Cantor, Dana Farber Cancer Center, Boston; D. Farber, UCLA, Los Angeles; D. Nebert, U. Cincinnati, Cincinnati; M. Brilliant, The Jackson Lab., Bar Harbor; J. Trapani, Sloan Kettering, NY; D. Joseph, U. NC, Chapel Hill; L. Old, Sloan Kettering, NY

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Viral Biology

INSTITUTE AND LOCATION
NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have recently expanded our efforts to chromosomally map genes in the laboratory mouse. Such studies have received increased interest through the Human Genome Initiative, which views the mouse as a unique model system. Our studies, traditionally based on the analysis of somatic cell hybrids to assign genes to chromosomes, have been expanded to include Southern blot analysis of DNAs from an interspecies cross. These studies have resulted in the chromosomal mapping of genes encoding zona pellucida proteins and enzymes, genes involved in drug metabolism, brain-specific genes, and numerous genes involved in visual transduction, including a cDNA which may be responsible for retinal degeneration, an inherited disorder.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00353-08-LMM

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Structural and Functional Studies of Mammalian Endogenous Retroviral Sequences

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. S. Khan	Microbiologist	LMM, NIAID
Others:	P. Kedar	Visiting Associate	LMM, NIAID
	R. Cuttler	Research Chemist	IRP, NIAID

COOPERATING UNITS (if any)

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Laboratory of Molecular Microbiology

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NIAID, NIH Bethesda, MD 20892

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☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Nucleotide sequence of the IN genes of leukemogenic mink cell focus-forming murine leukemia virus (L-MCF13 MuLV) and non-leukemogenic (NL) MCF111A MuLV was determined. The two IN genes were diverged in their 5′ sequences, whereas the 3′ portion was fairly conserved. The biological activity of MCF13 and MCF111A IN genes was studies using an *in vitro* constructed recombinant MCF13 DNA which contained MCF111A IN gene. The recombinant virus and parental viruses were compared in their leukemogenic potential and ratios of unintegrated and integrated proviral DNAs. The results indicated that the two MuLV IN genes were different in integrase activity.

Nucleotide sequence of the IN gene of an endogenous MCF MuLV-related DNA (B34) was determined. The gene was 1230 bp and could potentially encode for a functional protein. Comparative sequence analysis indicated that the endogenous IN gene had 82% sequence homology and 86% amino acid homology with AKV ecotropic MuLV IN.

In situ hybridization analysis indicated that human endogenous 4-1 retroviral-related RNAs were expressed at high levels in both human brain and kidney tissues, but only in a few cells.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00304-09 LMM

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Retroviral Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. Silver	Senior Investigator	LMM, NIAID
Others:	T. Jeang	Senior Staff Fellow	LMM, NIAID
	R. Repaske	Research Chemist	LMM, NIAID
	O. Nahor	Visiting Fellow	LMM, NIAID
	K. Fujita	Visiting Associate	LMM, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.6	2.6	0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We used polymerase chain reaction (PCR) to construct recombinants between related viruses in order to investigate structure-function relationships of viral genes. In collaboration with Stephen Feinstone (CBER/FDA), the VP4 gene of polio virus was substituted for the VP4 gene of hepatitis A virus (HAV). The resultant virus was non-infectious, suggesting virus-specific interactions between VP4 and other HAV genes. In collaboration with Kuan Teh Jeang, the SV40 enhancer was substituted for the HIV enhancer in an HIV-CAT construct. The resultant construct was only partially responsive to tat, implicating the HIV enhancer in the tat regulatory system. In collaboration with Roy Repaske, a chimeric MoMuLV-HIV reverse transcriptase (RT) gene was constructed. This gene has HIV-like rather than MoMuLV-like reverse transcriptase activity in bacterial cells, localizing these traits to the HIV substituted segment. With Orit Nahor I constructed a chimeric MoMuLV with the HIV U3 region substituted for the MoMuLV U3 region. This chimeric virus was non-infectious, suggesting that the HIV enhancer does not substitute for the MoMuLV enhancer in mouse cells. Recently, another MoMuLV chimeria was constructed in which the HIV tat gene, plus an upstream intron, was inserted into the MoMuLV U3 region. The chimeric virus expressed tat, but was non-infectious, while the same chimera with the insert in the opposite orientation did not express tat and was infectious. This chimera will be useful to study effects of splicing on viral infectivity.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00415-06-LMM

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Molecular Biology of Retroviruses Associated with AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. A. Martin	Chief	LMM, NIAID
Others:	K. Strebel	Senior Staff Fellow	LMM, NIAID
	L-J. Chang	Visiting Associate	LMM, NIAID
	K. Peden	Expert	LMM, NIAID
	R. Willey	Biologist	LMM, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH: Laboratory of Molecular Microbiology

SECTION: Biochemical Virology

INSTITUTE AND LOCATION: NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	3	3

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Processing of HIV envelope glycoproteins: The intracellular transport and processing of HIV gp160 has been investigated using wild-type and *env* mutants of molecularly cloned virus. Pulse-chase experiments in conjunction with a variety of biochemical analyses were conducted on cells acutely infected with virus stocks or transiently transfected with wild-type or mutagenized proviral DNA clones. These studies indicate that the intracellular transport and processing of the gp160 *env* precursor polyprotein proceed via the rough endoplasmic reticulum (ER) and the various Golgi compartments. The formation of oligomeric gp160 structures within the ER may be required for transport; however, appropriate folding of the oligomerized gp160 is critical for accurate glycosylation, cleavage, and incorporation into progeny virions.

Structure/function analyses of the HIV *vif* and *vpu* proteins: The HIV *vif* protein has previously been shown to be critical for virus-mediated infections. This 23 kDa protein is neither particle-nor membrane-associated and exists in cells in very low concentrations. The *vif* protein does not form oligomeric structures in infected cells, nor is it modified post-translationally. In contrast, large quantities of the HIV encoded *vpu* protein are synthesized during productive infection. *Vpu* is an integral membrane protein, is phosphorylated, and forms homopolymeric complexes, most likely tetramers, in infected cells. The interaction of *vpu* with other viral or cellular proteins is presently under investigation.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00437-06-LMM

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Biology and Genetics of the AIDS Retrovirus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. D. Hoggan	Senior Scientist	LMM, NIAID
Others:	G. Englund	Biologist	LMM, NIAID
	M. A. Martin	Lab Chief	LMM, NIAID
	C. R. Wood	Staff Scientist	MCG, Genetics Inst.
	P. Szklut	Biologist	MCG, Genetics Inst.

COOPERATING UNITS (if any)

Molecular and Cellular Genetics, Genetics Institute, Cambridge MA.

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.1	0.4

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the past year studies have concentrated on the production of broadly reacting antibody in mice, utilizing the principal of original antigenic sin (OAS). Antibody was produced in BALB/c mice by first priming them with purified gp120 from the LAV strain of HIV conjugated to KLH, followed by serial immunization with CEM cells optimally infected with four wildly divergent strains of HIV-I. These included the ΔU mutant clone of PNL4-3, p Alabama 15.3, RZ-34 MC, and wild-type Z84. Not only did these animals develop reactivity in the ELISA and RIP tests against gp120 (titers 1:60 $\rightarrow$ 1:1920), they developed neutralizing antibody (titers 1:240 $\rightarrow$ 1:1920) against the various strains to which they were exposed. In addition, they developed neutralizing antibody against the NYS strain of HIV (1:20 $\rightarrow$ 1:120) to which they had not been immunized. To date, one animal has been sacrificed and its spleen cells fused to P-3 myeloma cells. Approximately ten primary clones were picked and, after secondary cloning, four clones remain which demonstrate specific reactivity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00438-06-LMM

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Molecular Biology of Cellular Oncogenes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. Santos	Visiting Scientist	LMM, NIAID
Others:	T. Bryan	Microbiologist	LMM, NIAID
	A. R. Nebreda	Visiting Fellow	LMM, NIAID
	M. Benito	Guest Researcher	LMM, NIAID
	F. Segade	Guest Researcher	LMM, NIAID
	S. Venkatesan		LMM, NIAID

COOPERATING UNITS (if any)

George VandeWoude, ABL, BRI, NCI-FCRDC
Paul Wingfield, Office of the Director, NIH

LAB/BRANCH: Laboratory of Molecular Microbiology

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INSTITUTE AND LOCATION: NIAID, NIH Bethesda, MD 20892

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

G Proteins participate in the transduction of cellular signals by cycling between an active conformation (complexed with GTP) D and an inactive conformation (complexed with GDP). The *ras* proteins are guanine nucleotide binding proteins believed to participate in the transduction of proliferative signals, although their specific role is still unknown. Using a variety of experimental approaches, we showed that they are not regulatory elements of either of the two main signaling pathways in mammalian cells, namely the adenylate cyclase and phosphoinostitide pathways; however, using *Xenopus* oocytes, we were able to show that they exert a regulatory role (even in the absence of protein synthesis) in cascades of phosphorylation associated with the induction of proliferation and cell division. The HIV *nef* gene product has also been proposed to act as a regulatory G protein based on reports attributing GTP binding and GTPase activity to these proteins. We expressed different cDNA clones of *nef* using the same bacterial and mammalian vectors used previously for *ras*. Unlike *ras, nef* from the different HIV isolates lacked GTP binding activity but showed autophosphorylation using either GTP or ATP as the phosphate donor. In addition, unlike *ras*, HIV *nef* did not exhibit oncogenic potential in focus-forming assays with NIH 3T3 cells, nor did it cause meiotic maturation of *Xenopus* oocytes. It therefore appears that the biological function of *nef* does not follow the pattern of G proteins; however, its phosphorylation, either by protein kinase C or through its autokinase activity, may be functionally significant *in vivo*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00467-05-LMM

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Polypeptides Encoded by the HIV-1 Genome.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Sundararajan Venkatesan Expert LMM, NIAID

COOPERATING UNITS (if any)

M. A. Martin, Chief, LMM, NIAID; T. Fuerst, Staff Scientist, MVI, Gaith., MD; E. Ross, Microbiologist, LMM, NIAID; J. Orenstein, Pathologist, GWU Sch. Med., Wash, DC.

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In previous years, we have purified and sequenced HIV-1 p64 and p51 RT (reverse transcriptase) subunits, the p34 integrase, and the p10 *gag-pol* protease responsible for the processing of the *gag* and *gag-pol* fusion. A sensitive assay for the protease using defined oligopeptides was also developed. Further, a GAG precursor processing pathway that results in seven *gag* subunits was deduced. We also identified a novel *de novo* synthesized *gag* p41 species, initiating at an internal Met at position 142 of the *gag* ORF.

Recombinant vaccinia viruses expressing the HIV-1 protease, p64 RT, p55, and p41 *gag* precursor proteins with or without protease were constructed. Protein expression has been confirmed by immunological procedures. The integration protein was also expressed in the vaccinia vector. A 15 kD C-terminal cleavage product of the p64 RT was found to have RNAse H activity, and the DNA corresponding to this protein has also been expressed in vaccinia.

Using a vaccinia virus expression system, we demonstrated that self-assembly of p55 GAG protein is sufficient to form the framework of the nascent human immunodeficiency virus (HIV-1) particle. The particles which budded from the cells infected with a vaccinia-GAG construct were mostly spheres with a concentric ring of electron-dense material. Expression of the GAG and POL proteins resulted in mature particles containing a condensed core which assumed the nucleoid structure characteristic of lentiviruses. When the POL frame in the GAG-POL ORF was truncated at the end of the protease domain, p55 GAG processing was markedly reduced and the maturation of the resultant particles was defective.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AI-00527-03-LMM

PERIOD COVERED October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular Cloning and Characterization of Retroviruses Associated with AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T. S. Theodore	Research Microbiologist	LMM, NIAID
	M. A. Martin	Laboratory Chief	LMM, NIAID
Others:	K. A. Clouse	FDA	LMM, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH Laboratory of Molecular Microbiology

SECTION

INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

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☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A prominent feature of AIDS is the heterogeneity among HIV-1 retroviral genomes. Molecular cloning, restriction enzyme analysis, nucleotide sequencing, and infectivity studies have been used to characterized the various isolates. These studies provide information regarding their structural as well as geographic diversity and, possibly, the degree of antigenic variability and pathogenicity among different HIV-1 isolates. Virus prepared from the different molecular cloned HIV isolates are presently being used to prepare monoclonal antibodies to test their neutralization activity against a wide spectrum of HIV isolates.

A T cell clone (ACH-2) from lymphocytes infected with HIV-1 (LAV) produced HIV-1 in response to stimulation with phorbol myristic acetate (PMA). An isolate was molecularly cloned and, upon transfection into T cells, produced virus; however, only in the presence of PMA. Passage onto fresh lymphocytes produced virus as monitored by reverse transcriptase activity (RT) in the absence of the inducing PMA. Mixing and matching experiments swapping the *gag-pol-env* region between the ACH-2 clone and LAV parent clone, restored biological activity of ACH-2 to wild-type LAV. The ACH-2 isolate may provide an understanding of the mechanism of pathogenicity of HIV as it relates to chronic infections and/or latency and viral induction.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI-00528-03-LMM

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Functional Interactions of HIV Regulatory Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. Venkatesan	Expert	LMM, NIAID
Others:	N. Ahmad	Visiting Associate	LMM, NIAID
	R. K. Maitra	Visiting Fellow	LMM, NIAID
	S. M. Holland	NRC Res. Assoc	LMM, NiAID

COOPERATING UNITS (if any)
E. Santos, Visiting Assoc.,LMM,NIAID; P. Wingfield, Chief,Prot. Exp. Lab,OD,NIH; C.M. Lane, Med. Officer,LIR,NIAID; P. Dickie, Staff Fellow,LMM, NIAID; T.M. Fuerst, Staff Scientist,MVI,Gaith,MD; S. Koenig, Med Staff Fellow, LIR, NIAID.

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NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Replication of human immunodeficiency virus, type 1 (HIV-1) requires the function of a 21 kD nuclear protein, REV. REV mediates its function by interacting with a highly structured viral mRNA sequence, RRE (REV responsive element) of about 236 nt located in the ENV ORF and facilitates the nuclear export of viral mRNAs containing RRE. Purified REV bound RRE RNA in a sequence-specific manner. The critical functional elements of RRE fold into a structure composed of a stem-loop A formed by the ends of the RRE joined to a branched stem-loop B/B1/B2, between bases 49-113. Maintenance of the native RRE secondary structure alone was not sufficient for REV recognition. Mutations that changed a 5′..CACUAUGGG..3′ sequence in the B stem without affecting the overall structure abolished both the *in vitro* REV binding and the *in vivo* REV response. Large quantities of biochemically active REV (2-3 mg/ 10^9 cells) were also expressed using a TK-vaccinia virus recombinant. Increasing amounts of NEF induced dose-dependent repression of virus production from provirus correlating with a proportionate decrease of all viral transcripts. Transcription from the LTR devoid of sequences between 159 - 340 nts (referred to as NRE) upstream of the mRNA start site was no longer suppressed by Nef. Stable T lymphocyte and HeLa cell lines expressing NEF were also shown to down-regulated LTR transcription and HIV-1 replication. NEF expressing vaccinia virus recombinant was used to identify CTL response against NEF in 2 AIDS patients before onset of serological response to NEF or other HIV proteins. By peptide competition experiments, 2 major MHC class I restricted CTL epitopes were mapped. One, a 10-mer between residues 73 and 82, is a remarkably conserved domain among the different HIV isolates, though the overall sequence of Nef is highly variable. Six transgenic founder mice (FVB/N) bearing HIV-1 LTR-linked NEF were constructed. Adult transgenic mice developed persistent papillomatous skin lesions marked by follicular hyperplasia of the basal cell proliferation of the epidermis. Proliferating skin lesions expressed large amounts of NEF RNA and the 27 kD NEF protein. NEF mRNA and protein expression were absent or negligible in the unaffected regions of skin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00546-02-LMM

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Human Retrovirus Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. B. Rabson, M.D.		
Others:	T. J. Seidner	Guest Researcher	LMM, NIAID
	W. Turner	Biologist	LMM, NIAID

COOPERATING UNITS (if any)

Malcolm A. Martin, M.D., LMM, NIAID; Elizabeth Ross; Alicia Buckler-White, Ph.D., Georgetown University.

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project continues to be the understanding of the role of viral-cellular interactions in the regulation of gene expression of the human immunodeficiency virus (HIV). We have studied the role of *cis*-acting DNA sequences in the HIV long terminal repeat (LTR) in the control of HIV gene expression in both acute infection of human T lymphocytes and in chronic or latent infections. In the past year, our studies have focused on the Sp1 binding sites in the HIV LTR. Virus containing a deletion of all three Sp1 sites replicates efficiently in PHA-stimulated human peripheral blood lymphocytes (PBLs) and in the MT4 human T cell line, but did not replicate in A3.01 cells unless those cells were treated with the cytokine, TNF-α. Gel retardation assays were performed to study the basis for the differential replication of the Sp1-deleted virus. While both MT4 cells and A3.01 cells contained abundant Sp1 binding activity, NF-κB activity could be detected in the nuclei of MT4 cells, but was not present in A3.01 cells unless those cells were treated with TNF-α. Thus, the presence of NF-κB binding activity in the nuclei of target T cells appeared to be required for the replication of proviruses deleted in the Sp1 sites. This suggests that the NF-κB and Sp1 binding sites in the LTR share the properties of "enhansons" for HIV gene expression and may functionally substitute for each other in activating HIV replication. Recently we have identified revertants of the Sp1 deleted virus that have recovered the ability to replicate in A3.01 cells. These viruses retain the Sp1 site deletion but have an extra NF-κB binding site. The mechanisms by which a third NF-κB site now allows viral replication in A3.01 cells are currently under study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00547-02 LMM

PERIOD COVERED

October 1, 1989 to September 31, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanism(s) of Human Retrovirus *trans*-Regulatory Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K-T. Jeang	Sr. Staff Fellow	LMM, NIAID
Others:	B. Berkhout	Visiting Associate	LMM, NIAID
	A. Gatignol	Visiting Fellow	LMM, NIAID
	O. J. Semmes, IV	Adjunct Scientist	LMM, NIAID
	N. Nelson	Chemist	LMM, NIAID
	M. Martin	Chief	LMM, NIAID

COOPERATING UNITS (if any)

None

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INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	4	1

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have studied the mechanism of action of the HIV-1 Tat, HTLV-I Tax, and HTLV-I Rex proteins. We have made the following salient observations: 1) HIV-1 Tat acts as a transcriptional *trans*-activator of LTR-directed expression. Tat uses a bipartite target that includes both a nascent RNA and specific DNA elements within the LTR promoter. 2) The HIV-2 Tat protein acts like HIV-1 Tat, except that it requires a duplicated TAR RNA secondary structure for optimal activity. 3) A cellular protein that binds with
specificity to the HIV-1 TAR RNA appears to be a general transcriptional factor. 4) HTLV-I Tax has pleiotropic up- and down-regulatory activities. Mutiple protein domains within Tax are apparantly necessary to mediate these diverse functions. 5) A novel transcriptional function for HTLV-I Rex has been observed.

Our current understanding is that modulation of the HIV-1/HTLV-I LTR is mediated through complex interactions between cellular transcription factors and the viral *trans*-regulatory proteins.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00588-01-LMM

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of Simian Immunodeficiency Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. S. Khan	Microbiologist	LMM, NIAID
Others:	T. Galvin	Guest Researcher	LMM, NIAID
	L. Lowenstine	Assoc. Professor	Univ. of Calif. Davis

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

SIVstm was isolated from a rhesus macaque which developed AIDS-like disease upon inoculation with lymph node homogenate of a stump-tailed macaque which had died from a lymphoma. A 2.0 kb fragment containing the 5′ LTR and *gag* region of SIVstm was amplified by PCR from virus-infected human PBL DNA. Nucleotide sequence comparison of the *gag* regions of SIVstm and other SIVs indicated that SIVstm had 84% homology to SIVs isolated from Asian pig-tailed (SIVmne) and cynomolgous (SIVmac) macaques and African sooty mangabey (SIVsmm). SIVmne and SIVmac (which are 96% homologous to each other and 87% to SIVsmm) are thought to have originated in captivity by cross-infection of Asian macaques by SIV of sooty mangabey. The nucleotide sequence divergence of SIVstm from the other SIVs suggests that stump-tailed macaques were infected with a virus different from that of the sooty mangabey.

PHS 6040 (Rev. 1/84) GPO 914-918

LABORATORY OF PARASITIC DISEASES
1990 Annual Report
Table of Contents

Laboratory of Parasitic Diseases
National Institute of Allergy and Infectious Disease
Summary - October 1, 1989 - September 30, 1990

ADMINISTRATIVE AND ORGANIZATIONAL EVENTS

LPD has pretty much adjusted to their new home in Building 4 and even some of the engineering defects appear to have been corrected. We appreciate the help of the Intramural administrative staff. Now, if there were only some way to get additional space!

A considerable turnover in professional personnel continued as usual. Three members of the Malaria Section's entomology group left to take positions elsewhere: Klaus-Peter Sieber returned to West Germany, Cecilia Graziosi moved over to LIR in our Institute to work on AIDS, and Pat Romans left for an academic position at McGill University, ~Montreal in her native Canada. In Alan Sher's Section, Stephen Heath returned to England for an academic job, and Mathilde Knight took a position with the Biomedical Research Institute in Rockville. Even though he was with LPD for only one year, Bruno Gottstein from Ted Nash's lab impressed us greatly with his hard work and efficiency--he returned to his position at the Institute of Parasitology in Zurich, Switzerland. Ajit Limaye, the Howard Hughes Fellow, returned to finish Medical School in Seattle after a two year virtuoso research performance in Eric Ottesen's lab. Two Medical Staff Fellows finished up--Michael Ballard, whose plans for next year are not yet settled, and Beverley (Lee) Hall, who spent his last year with Keith Joiner at Yale, will continue as a post-doc at that laboratory.

The Malaria Section received a heavy infusion of Guest-Investigators who came with financial support outside of NIH. On WHO grants are Wendy White, a recent Ph.D. from Georgetown University to work with Dr. McCutchan, and Kim Williamson from NHLBI to work with David Kaslow. Dr. Paulo Pimenta is a cell biologist supported by a grant from the McArthur Foundation to work on both sandfly vectors with David Sacks and anopheline vectors with the Malaria Section. Another Brazilian, Dr. Antoniana Krettli came with a Brazilian fellowship (CAPES) to work on immunology of malaria with Drs. Kumar and Miller. Hedvig Perlman is back after she and her husband, Fogarty Scholar Peter, went back to Sweden for a few months. Kathleen Creedon is a Guest Researcher working for the FDA, but also on her Ph.D. from Catholic University. Dr. Sher's Lab of Immunology and Cell Biology also had a number of newcomers. Victoria Mann, a recent Ph.D. From University of North Carolina came to work with Dr. Dwyer as an IRTA Fellow and Cheryl Champion, a recent Ph.D. from UCLA came to work on schistosome antigens with Dr. Sher.

Physicians Christopher Karp and Siddhartha Mahanty, joined us in the past year under the National Research Service Award (NRSA) program for research training. Dr. Karp comes from the Georgetown University residency training program to work with Drs. Sacks and Neva on leishmaniasis, and Dr. Mahanty from Western Reserve joins Ottesen's group.

We also have had numerous Guest Researchers who came for shorter periods, Generally 3 to 6 months, for special work under non-NIAID support. These include Dr. Nahid Ali from Calcutta, India and Elvira Saravia from Brazil, both of whom worked with Dr. Sacks on leishmaniasis. Luis Andrade from Recife, Brazil, working in Ottesen's lab will continue to participate in the collaborative program between LPD and Recife on filariasis. Dr. Sudha Bhattacharya made her last visit from India under the special Rockefeller Foundation fellowship to work with Dr. Diamond on amebiasis. Her husband, Alok, will come next year.

As usual we will report only the foreign travel of LPD staff for research work (TDY); foreign travel to meetings is too complicated to document because it is so frequent and under such varied sponsorship. As usual, Drs. Ottesen and Nutman and coworkers on their filariasis projects led the list. In the process of assisting WHO to initiate treatment trials with Ivermectin for filariasis in India, Malaysia, Brazil, and Tahiti, Dr. Ottesen also helped members of his lab (Dr. King, Ajit Limaye and Bob Poindexter) get their research started in India. He also was able to check on the filariasis situation in the Cook Islands in the Pacific, where he had worked in the mid 1970's. Dr. Nutman made several trips to Guatemala, once with Kathy Steel and once with Dr. Lobos, where they are winding up their studies of onchocerciasis. In addition, Drs. Nutman and Klion spent about 3 weeks in Benin, West Africa, where a very useful interaction has been established to examine the effects of *Loa loa* infection on an endemic population at a palm oil plantation and compare them to the expression of disease in U.S. Peace Corps. volunteers. Drs. Dolan and Peterson from the Malaria Section spent about 10 days in Belem, Brazil collecting specimens and working with local colleagues on the problem of drug resistance.

HONORS AND AWARDS

All of the permanent professional staff, and many of the senior nontenured staff serve on Editorial Boards of journals and frequently review manuscripts for journals. Clearly this activity is evidence of the high standing of LPD scientists by professional peers. Also, many of the Staff serve on Ad Hoc Committees for granting agencies or foundations, and are invited to participate in workshops and scientific meetings. Such activities are not cited individually here, unless they deserve special mention.

The most prestigious recognition of an LPD staff member in the past year was the election of Dr. Louis Miller to the National Academy of Sciences. Dr. Miller was also elected to the Association of American Physicians.

LPD had the unusual distinction of having two of its senior staff, Drs. Eric Ottesen and Alan Sher as recipients of the Bailey K. Ashford Award of the American Society of Tropical Medicine and Hygiene. This is customarily given to a single person 45 years or less of age every three years for recognition of their research work.

Dr. Thomas Nutman was recipient of a PHS Achievement Medal, and we were pleased to be able to have him converted to the status of tenured investigator in LPD.

RESEARCH PROGRESS

The Malaria Section

MOLECULAR GENETICS OF MALARIA PARASITES

Search for chloroquine resistance gene continues. Chloroquine resistance is associated with rapid loss of the drug from acid vesicles containing the parasites. Since this process of drug efflux is similar to that occurring in multi-drug resistant (mdr) tumor cell lines, and because *P.falciparum* also contains several mdr-like genes, they have been implicated as responsible for chloroquine resistance. However, linkage analysis of products of the genetic cross between chloroquine resistant and chloroquine sensitive clones of *P. falciparum* does not support this hypothesis. Examination of 16 recombinant progeny of the cross indicated that the rapid efflux phenotype was controlled by a single gene or closely linked group of genes. There was no linkage between the rapid efflux resistant phenotype and either of the mdr-like genes from *P. falciparum* or amplification of those genes. Thus, the search for the chloroquine resistance gene continues (Wellems, Walker-Jonah, Creedon).

Use of PCR to map malaria resistance to anti-fols. As reported last year, the point mutation in *P. falciparum* strains resistant to proguanil was found to involve amino acid substitutions at positions 16 and 18 in the dihydrofolate reductase (DHFR) molecule. Resistance to pyrimethamine was found to involve only a single site at position 108. However, in a few strains changes at positions 108 and 164 are associated with resistance to both anti-fols (Peterson and Wellems). Practical application of this information will be sought by field studies in Brazil from blood specimens of *P. falciparum* infected patients collected in different areas of northern Brazil and sent to Belem. By appropriate use of primers and oligonucleotides for the polymerase chain reaction (PCR), the DHFR molecule in the blood specimens will be amplified to determine whether it is the sensitive or resistent pattern. In this way the drug sensitivity to these anti-fol drugs can be mapped as a guide for prophylaxis or treatment (Wellems, Dolan, Peterson and Brazilian coworkers).

Molecular switching enables Parasite to invade. A clone of *P. falciparum* resulting from a genetic cross was found to invade neuraminidase treated RBCs at a greatly reduced rate without need for sialic acid residues on the membrane that normally are required. A line of this Dd 2 clone now invades either normal or sialic acid deficient RBCs at the same rate. It is hypothesized that a switch in gene expression by the parasite occurred, activating a nonsialic acid dependent pathway. A search is underway to identify the molecular basis for this switching phenomenon (Dolan and Wellems).

Duffy receptor for P. *vivax* cloned. Red cells containing Duffy blood group determinants are essential for invasion by both *P. vivax* and *P. knowlesi* merozoites; hence, portions of the Duffy antigen are considered to be receptors for the invading parasite. Last year it was reported that a gene encoding a member of the Duffy receptor gene family of *P. knowlesi* had been cloned. Using the *P. knowlesi* gene as a probe, the Duffy receptor gene for *P. vivax* was cloned. Considerable information on the amino acid sequence of both the *P. vivax* and *P. knowlesi* Duffy receptor genes, including functional binding domains, is now available (Fang, Kaslow, Adams Miller).

APPROACHES TO MALARIA VACCINES

T cell responses alone Generally not sufficient for immunity to rodent malaria. With several species of rodent malaria *P. vinckei, P. yoelii* and *P. berghei)* infection and cure results in solid immunity to reinfection. Adoptive transfer of CD4+ T cells reconstitutes immunity in T cell depleted mice, but splenectomy, even of immune mice, results in loss of immunity. Immunization of animals with killed *P. vinckei* plus various adjuvants failed to protect mice from live challenge, but killed *P. vickei* in combination with infection of attenuated Salmonella typhimurium induced a high degree of protective immunity. These results suggest that effective immunity requires both a malaria-specific CD4+ T cell response as well as certain functional changes in the spleen. A novel approach is planned to screen recombinant clones of *P. vinckei* in live attenuated Salmonella for protective antigens in mice. Clones that show protective activity will then be analyzed to identify the protective antigen (Kumar, Hedvig and Peter Perlman, Miller, and Krettli).

The 25 kDa sexual stage *P. falciParum* antigen hangs tough! Heroic efforts have been expended upon developing this surface antigen on ookinetes to the point of a suitable transmission blocking vaccine candidate. When cloned and expressed in bacterial vectors, some molecular "glitch" (presumably interference with bacterial folding) leads to suboptimal antigenic activity. When expressed in mammalian cells with a recombinant vaccinia vector, the 25kDa protein is a better antigen. (Kaslow and Moss of LVD) The possibility that several different epitopes on the 25kDa protein may be targets of transmission blocking antibodies and interfere with each other is also being explored. Analysis of immunogenicity of overlapping peptides of the 25kDa protein for both T and B cell epitopes has been interesting, but not particularly helpful. (Kaslow and Quakyi). Finally, cloning of analogous genes to the 25kDa protein in other species of malaria parasites with oligonucleotide probes is being attempted. The possibility of help from biotechnology companies to obtain more effective expression of the 25kDa protein in other systems is being sought. (Kaslow, Miller and various commercial collaborators)

Other transmission blocking antigens. The possibility of developing other antigens on sexual stages of the malaria parasite as vaccine candidates is also being explored. The 40kDa protein has been characterized further, and a polyclonal antibody with good reactivity has been produced, with prospects that a monoclonal antibody will also be available. Work is being resumed on the 48/45 kDa and 230 kDa antigens which have been previously described (Rawlings and Kaslow). A new procedure for obtaining better yields of *P. falciparum* microgametes has been developed which may be useful not only to search for new vaccine candidate antigens but also define requirements in the fertilization process. With a reproducible and simplified fertilization assay it might be easier to identify compounds that inhibit fertilization (Keister and Kaslow).

Glucose-6-phosphate dehydrogenase (G6PD) and malaria. It has been hypothesized that G6PD deficiency protects the female heterozygote from malarial mortality. *P. falciparum* parasites contains their own G6PD but it is not clear as to whether it requires several cycles of growth before it is induced. A strategy for cloning the gene for parasite G6PD is being tested, and the importance of G6PD deficiency in protection vs. malaria will be tested in mice deficient in this enzyme (Kaslow).

GENETIC MANIPULATION AND PHYSIOLOGY OF VECTORS

In-situ hybridization of mosquito chromosomes. Mapping of chromosomes has been a basic ANOPHELINE technique in *Drosophilia* genetics, and for this polytene chromosomes (giant chromosomes that occur in nuclei of certain somatic tissues of *Diptera)* are studied. Methods used for polytene chromosomes of *Drosophilia* salivary glands had to be modified so in-situ hybridization could be applied to polytene chromosomes from ovarian nurse cells of *Anopheles gambiae*. Using this procedure, unique DNA sequences from a genomic library of *A. gambiae* have been mapped. Such maps will facilitate identification of genetic loci and be a useful tool for genetic studies of *A. gambiae* on a molecular level. It was of particular interest that the construct containing the neomycin resistance gene (pHS neo), injected into *A. gambiae* eggs and stably integrated into progeny, was identified by in-situ hybridization on the telomere of chromosome 2L (left arm of 2) Graziosi, Sakai and Romans).

Effects of antibody on malaria parasite in the mosquito. The point at which a monoclonal antibody (Mab) to a surface protein on *P. gallinaceum* ookinetes (analogous to the 25kDa of *P. falciparum)* blocks development of the parasite in *Aedes aegypti* was examined. First, it was shown that antibody acted only on ookinetes, not on the earlier zygote stage. Ookinetes attached to the peritrophic membrane (which forms in the stomach after a blood meal) whether incubated with the Mab or not. But two different populations of mosquitoes were subsequently observed--one subpopulation with low numbers of oocysts per midgut indicating an effect of the antibody, and another subpopulation with large numbers of oocysts per midgut, indicating failure of antibody to block invasion of the midgut epithelium. While a definitive explanation for this inconsistent action of the antibody on development of the parasite in the mosquito is not possible, the results are interpreted as reflecting variations in the integrity of the peritrophic membrane to the parasite. In other words, sometimes the antibody blocks ookinete penetration of the membrane and sometimes it does not (Sieber, Miller and Sakai).

RIBOSOMAL RNA OF MALARIA PARASITES

Use of ribosomal RNAs (rRNAs)to assay exo-erythrocytic development of Parasites. As reported last year, two distinct rRNAs characterize the sexual and asexual stages of malaria parasites, respectively. The transition from one type to the other occurs shortly after sporozoites invade hepatocytes. Invasion itself is not sufficient to trigger the rRNA transition, but even irradiated sporozoites initiate RNA synthesis, at least for a time. By use of a susceptible cell line the rRNA initiated from as few as 10-50 sporozoites of *P. berghei* can be detected by RNA dot blot analysis with a single oligonucleotide probe. Since an actual increase in RNA can be demonstrated over the period of exoeryrocytic (EE) development, such an in-vitro system may be useful in screening drugs for activity against EE stages of the malaria parasite (McCutchan, Waters and BRI collaborators).

Phylogenetic analysis of *Plasmodium* species via rRNA genes. By biologic and morphologic criteria *Plasmodial* species were initially classified into mammalian and avian subgenera. Then, as molecular technology evolved, the GC content of genomic DNA suggested that the human parasite, *P. falciparum,* was very similar to both rodent and avian parasites.

Now, it is possible to carry out phylogenetic analysis of *Plasmodium* species from DNA sequences of small subunit rRNA genes. Such a comparison confirmed three major sub-groups, avian, simian and rodent. *P. falciparum* clustered with the avian subgroup and *P. vivax* resembled the simian group. (Waters, McCutchan and Higgins of Trinity College, Ireland).

Clinical Parasitology Section

IMMUNOREGULATION OF B AND T CELL RESPONSES

Cytokine control of eosinophilia. The cytokines IL-S, GM-CSF, and IL-3 have been implicated as being responsible for eosinophilia in humans. Conditions required to induce these cytokines by exposure to mitogens and antigens and to assay their presence were established. IL-S was shown to be produced in greater amounts by lymphocytes of patients with eosinophilia than those without eosinophilia. Also, IL-S could be induced by parasite antigen in patients previously sensitized. In addition, using both in-situ hybridization and the Elispot test the numbers of IL-S producing T cells were found to be significantly increased in patients with eosinophilia. After treatment of filarial infections, serum IL-5 levels were shown to precede the rise in peripheral eosinophil levels. Of interest is the preliminary observation that the eosinophilia seen in the eosinophilia-myalgia syndrome associated with tryptophan ingestion does not have the same IL-S dependence as seen in parasitic infections. (Limaye, Nutman, King and Ottesen)

Cytokine control of IgE response. Individuals with elevated serum IgE levels were found to have 10 to 100 fold more B cells committed to IgE production than normal individuals when assayed by the Elispot technique. Using parasite antigens in vitro IL-4 and also IL-6 were found to be produced, indicating that these cytokines are responsible for the B cell IgE response. It will be recalled that last year INF-gamma was found to down regulate the IgE response (King, Nutman and Ottesen).

Late response of IgG4 antibodies. The development of different isotypes of antibody after stimulation with protein antigens is known to follow a certain sequence--i.e., IgM first, followed by IgG antibodies. There is less information on the sequence and stimulus for the response of various IgG subclasses. Antibodies of the IgG4 subclass are known to be prominent in certain longstanding helminth infections, such as schistosomiasis and filariasis. They also have been implicated in blocking immediate hypersensitivity responses. The availability of serial serum samples over a year or more from patients with acute schistosomiasis provided a unique opportunity to examine the IgG4 subclass response. It was found that anti-parasite antibodies of the IgG4 subclass did not develop until 6 to 9 months after schistosome infection. So far as we know this is the first time that natural development of an IgG4 response in humans has been recorded. (Ottesen and Andrade).

IMMUNE RESPONSE TO FILARIAL INFECTIONS

Identification of protective antigens. The strategy of screening sera from infected and putatively immune individuals by Western blotting vs. parasite antigens has been used to identify protective antigens. Several observations suggest that antibodies against the infective larval stage might be more effective than adult worm antigens in preventing infection. Since infective larvae of the human *Onchocerca* parasite are not available, larvae from *Brugia malayi* and an animal *Onchocerca (O. lienalis)* were used.

Several proteins recognized by both IgG and IgE antibodies have been identified for further study. Also, genomic expression libraries have been screened to look for recombinant antigens (Lobos, Nutman and Ottesen). Similarly, sera from an endemic population for W. *bancrofti* infection from the Cook Islands were immunoblotted with larval antigens. A 43kD larval antigen recognized by infection-free cases. This antigen has now been cloned and the sequence of the gene is being determined. (Raghavan and Ottesen).

Loa infection in endemic vs. acutely exposed patients. Newly exposed patients, such as Peace Corps volunteers, who are exposed and become infected with *Loa loa* often show evidence of extreme immunologic hyper-responsiveness with high antibody titers, high IgE levels and marked eosinophilia. They are rarely microfilaremic and often have clinical manifestations. It was possible to carry out studies on about 100 patients living in a *Loa* endemic area in Benin West Africa for comparison. The endemic population, chronically exposed to *Loa* infection show relatively low lymphocyte proliferative responses and levels of antibody to filarial antigens, and only modest eosinophil responses--all evidence of marked down-regulation of immune responses (Klion, Nutman and Ottesen).

Treatment of lymphatic filariasis with Ivermectin. For many years diethylcarbamazine (DEC) has been the only practical treatment for *W. bancrofti* infections. However, there have always been compliance problems with DEC because (l) it must be given for several days, (2) it produces significant side effects, and (3) microfilaremia begins to recur after 6 months. Over the last 4 years controlled trials in several areas of the world have demonstrated that Ivermectin, over a broad dose range as low as 10 micrograms/kg up to 120mcg/kg, is equally effective as DEC in eliminating microfilaremia. However, the 6 months microfilarial recurrence rate is considerably greater with Ivermectin. But, since frequency and severity of side reactions were much lower at the 10 microgram dose with efficacy equal to that at higher doses, it now appears that low doses of Ivermectin at 10 or 20mcg/kg, followed by a later high dose without additional side effects, can keep microfilarial rates very low for more than a year. The big advantage of Ivermectin is that it can be given in a single oral dose. These studies illustrate how well-designed and controlled clinical treatment trials of parasitic infections can provide basic information of enormous practical importance (Ottesen, Merck Sharp and Dohme, and collaborators in India and Brazil).

Immunology and Cell Biology Section.

NEW APPROACHES TO VACCINES FOR SCHISTOSOMIASIS

Development of a paramyosin BCG recombinant. Paramyosin, a subtegmental constituent of schistosome worms, was previously shown to be reasonably effective as an experimental vaccine for schistosomiasis by inducing cell-mediated immunity when given with the adjuvant, BCG. In a series of novel molecular maneuvers the paramyosin gene was engineered into BCG organisms and has been shown to be expressed by Western blot analysis. Three different sized cDNAs of the cloned paramyosin gene were first ligated into a heat shock protein promoter site on a specially constructed plasmid. The plasmid was grown in *E. coli* then transfected into BCG. The paramyosin-BCG recombinant is being tested in mice to see if it can induce an anti-paramyosin immune response (Sher and Pierce in collaboration with de la Cruz of Molecular Vaccines, Inc.).

Screen of a cDNA library of parasite clones for reactive T-cells. A more empirical approach to detect new antigens which might induce cell-mediated immunity and protection is to screen recombinant expression products against T-cells from vaccinated mice. A lambda GT-ll adult worm cDNA library was first screened with a mixture of polyvalent antisera against different parasite preparations. The approximately 500 positive clones so obtained will

be screened for ability to elicit proliferative and INF-gamma responses in T-cells from mice immunized with irradiated cercariae (Sher, Pierce, Champion and F. Lewis of BRI).

Interplay of cytokine responses in experimental schistosome infections. Last year it was reported that mice immunized vs. schistosomiasis produced INF-gamma and IL-2, features of the Th 1 response, while the Th-2 response (production IL-4 and IL-S) was characteristic of infected mice. It has now been found that schistosome eggs are the major stimulus for the Th-2 response observed in infected mice. In fact, injection of eggs into vaccinated mice caused a complete suppression of their preexisting IFN-gamma response. A new cytokine, IL-10, has recently been described which down regulates production of Th-2 type cytokines (IL-4 and IL-S). How this all fits together in the immune response and immunopathology of this disease in humans is still pretty confusing! (Sher, Pearce, James, Gryzch and Cheever).

MOLECULAR BIOLOGY OF SCHISTOSOMES AND T. cruzi

Genetic analysis of natural schistosome populations. A cloned fragment of the ribosomal gene of *S. mansoni* was used in Southern hybridization analyses to look for genetic variation in natural populations of *S. mansoni*. Genomic DNAs were isolated from parasites from infected patients and from different geographic locations in Brazil. The genomic profiles of parasite DNA when probed with the ribosomal clone indicated that the Brazilian strains were more similar to each other than to Puerto Rican strains. Also, parasites from southeastern Brazil were more closely related to each other than they were to strains from northeastern Brazil. It was of interest that there was more variation within a natural population of strains isolated from snails than exhibited by strains maintained in the lab. (Brindley, Sher, Lewis of BRI and Correa Oliveira and L. Viera of Fio Cruz, Brazil).

Decay accelerating factor (DAF) of *T. cruzi* cloned. DAF, a substance produced by mammalian cells to prevent autologous complement activation via the alternate pathway, was previously shown to be present in trypomastigote stages of *T. cruzi* Mice were immunized with the *T. cruzi*-DAF material to produce polyclonal and monoclonal antibodies which inhibited Tc-DAF activity. The antibodies were also used to screen a lambda Gt-11-cDNA expression library prepared from mRNA of metacyclic trypomastigotes. Thirteen positive clones were isolated, of which five were chosen for purification and further study, (Sher, Kipnis and collaboration with Joiner and Ogden at Yale).

IMMUNE REGULATION OF EXPERIMENTAL TOXOPLASMA INFECTION

T-cell types involved in maintenance of immunity in mice. Infection with *Toxoplasma gondii is* common and generally asymptomatic in humans and most animals. However, with compromise of the immune system, as with AIDS, the parasite becomes activated, disseminates and can cause fatal disease. Cell-mediated immunity, and especially activity of INF-gamma is considered critical in maintaining resistance against the parasite. By in-vitro and in-vivo T-cell depletion experiments in mice it was concluded that Cd4+ lymphocytes play a synergistic role in vaccine-induced immunity, probably through the augmentation of IFN-gamma production by CD8+ effector cells (Gazzinelli, Sher and Hakim and Shearer of NCI).

BIOCHEMISTRY OF LEISHMANIA

Identification of amino acid and nucleoside transport proteins on leishmanial surface membranes. Previous work has demonstrated that proline and methionine are essential, growth limiting amino acids for leishmanial promastigotes, and that they are actively accumulated via high-affinity transport systems. Several unique radiolabled, substrate specific compounds were developed which were able to label, in situ, the proline and methionine transport proteins on membranes of viable L. *donovani* organisms. Such labeling was competitively inhibited by

>80% in presence of excess unlabeled compound. The transport proteins are composed of two externally oriented polypeptide subunits of about 54 and 56 kDa. Reagents are being developed for further characterization. Similar methods were used to identify an adenosine transport protein of approximately 33 kDa. (Dwyer and Mann).

Further studies on leishmanial secretory acid phosphatase (SAcP). Leishmanial SAcP was shown to possess a common antigenic epitope with parasite lipophosphoglycan (LPG), a functionally active surface molecule involved in serum complement interaction with the parasite. Both SAcP and LPG were shown to be processed in the Golgi compartment of the organism. (Dwyer). DNA probes have been developed to identify SAcP genes and genes responsible for LPG (Dwyer, Mallinson and Mann).

LEISHMANIAL CELL BIOLOGY AND IMMUNOLOGY

The role of LPG in developmental biology of leishmania. Attention continues to be focused upon the major surface molecule, lipophosphoglycan (LPG) on flagellate forms of leishmania. The LPG undergoes chemical and structural changes as the parasite develops to the infective stage so that it is resistant to serum complement. However, the developmental biology of New World leishmanial species appears to be different from *L. donovani, L. major* and *L. tropica* of the Old World. Although the LPG from New World species exhibit chemical alterations as they develop from log to stationary phase, the molecule does not appear to elongate, and the metacyclic promastigotes continue to be susceptible to serum complement (Sacks, Brodin, Pimenta and Turco of University of Kentucky). A direct agglutination test for Kala-azar with *L. donovani* promastigotes was found to require a mutant strain lacking LPG. The implications of this and utilization of the test is being investigated further (Sacks and Karp).

T-cell responses in human leishmaniasis. Further experience has been obtained with the system for examining T-lymphocyte responses after interaction with leishmania infected autologous macrophages or monocytes. The lymphocytes from 8 patients with Old World cutaneous *L. major* infections showed strong proliferative responses under these conditions. Cytokines (INF-gamma, IL-4, and GMCFS) produced by unfractionated or subpopulations of T-cells were also assayed in this system. New World patients with cutaneous leishmaniasis seem to be less dependent than Old World cases on CD4 cells for cytokine production. cDNA probes and reagents for cytokine assays have been developed and are now available for study of patients with leishmaniasis (Sacks, Cooper, Karp and Neva).

Host-Parasite Relations Section

CYTOKINES AND SCHISTO PATHOLOGY IN MICE

Ablation of eosinophils with anti-IL-5 on *S. japonicum* infected mice. Last year the effect of ablating eosinophil response in mice infected with *S. mansoni* by administration of anti-IL-5 monoclonal antibody was reported. The same procedure in mice infected with *S. japonicum* led to the same result--namely, no effect on egg laying or egg excretion, and only a minimal decrease in size of egg granulomas in the tissue (Xu and Cheever). Although not related to cytokines, another project has been evaluating long term fecundity of single pair *S. mansoni* or *S. Japonicum* infections in mice. Marked variation in numbers of eggs laid and numbers in the tissues was found with both species. Fecundity of *S. mansoni* does not change significantly up to 1 year after infection, but it does decrease with *S. japonicum*, even though worms do not die (Cheever and Mosiman of DCRT).

MOLECULAR BIOLOGY AND IMMUNOLOGY OF GIARDIA

Variation in the surface antigen. Studies have continued on the very complicated issue of surface proteins on the parasite which undergo antigenic variation as the parasite is grown. The surface antigens are cysteine rich proteins (CRP), so variation can be followed by evidence of transcription of the CRP gene and by monoclonal antibody reactivity of the surface antigen. Changes in surface protein expression by *G. lamblia* are accompanied by transcriptional activation of a new CRP gene, with concomitant arrest of transcription of a previously expressed CRP gene (Nash, Mowatt, and Nguyen).

Rates of antigenic variation and susceptibility to intestinal proteases. In cloned populations trophozoites with a new variant antigen were detected every 12-13 generations in isolate WB, and in every 6.5 generations in isolate GS. (Nash, Banks and Alling of OSD) Isolates and variants of *Giardia* differed in their susceptibility to intestinal proteases--some were totally resistant (Nash and Mowatt).

Physiology and Biochemistry Section

DNA of *T. CRUZI*

Differences in DNA of *T. cruzi*. Previous work has clearly established that different strains of *T. cruzi* may have quite different DNA content, and that this variability in DNA content per cell can be present among different clones of the same strain. Now marked interdevelopmental stage differences have also been found at the DNA level. In some clones the DNA per cell of epimastigotes may be the same as in trypomastigotes, but in other clones the DNA of these stages may be quite disparate. A second instance was found in which a cloned stock of Y strain *T. cruzi* developed a mixed population based upon DNA content of the cell. Environmental stress, such as change in temperature or nutrients, can also result in changes in parasite DNA (Dvorak and Nozaki). The methods used to study DNA content rely heavily upon flow cytometry instrumentation. In order to increase sensitivity of the instrumentation it was necessary to redesign hardware and develop new analytical software (Dvorak of LPD, Banks of OSD and Mudd of BEIB).

Anti-trypanosomal factor. Continued studies on purification of the anti-trypanosomal factor from *Pseudomonas* using HPLC and NMR disclosed that purified samples were contaminated with Tris buffer. Removal of the Tris has facilitated the purification process (Mercado with collaboration of Fales of NHLBI and Hammer of Georgetown Univ.).

Parasite Growth and Differentiation Section

MOLECULAR BIOLOGY AND BIOCHEMISTRY OF AMEBAE

"Riboprinting" differentiates Entameba species. By PCR amplification of a small subunit ribosomal RNA gene, and preparation of RFLPs from the amplified DNA, it is possible to rapidly identify and differentiate species of *Entameba*. When this technique of riboprinting was applied to the controversial issue of pathogenic (P) and non-pathogenic (NP) strains of *E. hystolytica* it was found that the organism is one species with two genetically distinct but interconvertible forms. In other words, riboprints from NP strains are different from P strains, but isoenzyme conversion from NP to P or vice versa does not change the ribosomal RNA gene patterns. The *E. histolytica*-like amebae that survive hypotonic media and grow at room temperature were identified by riboprinting as a sub-group of the genus *E. moshkovski*, a species considered to be free-living. Finally, strains of Entameba recovered from the uterus of IUD users were identified as *E. gingivalis*, a parasite of the oral cavity. (Diamond, Clark and Mirelman of Israel). By using monoclonal and polyclonal antibodies vs. various surface components of *E. histolytica*,

evidence has been found suggesting that bacteria can modulate surface antigens of amebae. (S. and A. Bhattacharya, Diamond, and Mirelman of Israel).

Respiratory metabolism of *Entameba* species. An unusual stimulatory effect of Mn++ ion was found on NADPH oxidase of *E. invadens,* a reptilian ameba. Other species of amebae failed to show this stimulatory effect of Mn++ (Weinbach and Diamond).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00094-31 LPD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Entamoeba histolytica: Molecular taxonomy and genetic mechanisms of virulence.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L.S. Diamond	Section Head	LPD, NIAID
Others:	C. G. Clark	IRTA Fellow	LPD, NIAID
	A. Bhattacharya	Guest Worker	LPD, NIAID
	S. Bhattacharya	Guest Worker	LPD, NIAID
	E.C. Weinbach	Section Head	LPD, NIAID

COOPERATING UNITS (if any)

Department of Biophysics, Weizmann Institute of Science (David Mirelman).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Parasite Growth & Differentiation

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A riboprinting technique based on the polymerase chain reaction and restriction fragment length polymorphism has been developed allowing positive identification of *Entamoeba* species. Using this technique we have confirmed the tentative identification of an *Entamoeba* found in the uterus of 1% of surveyed IUD users as *E. gingivalis*, a parasite of the human oral cavity.

The question whether nonpathogenic (NP) and pathogenic (P) strains of *E. histolytica* (based on isoenzyme and repetitive DNA characteristics) constitute one or two species has been addressed. *In vitro* conversion of NP strains to P characteristics suggest there is only one species with two forms. We have found that riboprints of NP strains differ from those of P strains. However, riboprints of converted strains are indistinguishable from one form before, and the other form after conversion. This implies that *E. histolytica* is one species existing in two genetically distinct but interconvertible forms.

Entamoeba histolytica-like isolates from humans, although different physiologically and biochemically from *E. histolytica* have been used in research as examples of avirulent forms of the latter. Riboprinting clearly indicates that *E. histolytica*-like amebae belong to a sub-group of *E. moshkovskii*, a species ordinarily isolated from sewage and considered to be free-living.

Monoclonal and polyclonal antibodies developed in this laboratory were used to monitor differences between xenically and axenically cultured *E. histolytica*. Using monoclonal antibodies 2D7.10, 2F3.4, and a polyclonal antibody, 1125, which recognize a carbohydrate antigen, a polypeptide antigen, and hydrophobic membrane components respectively, we have obtained evidence suggesting bacteria can modulate surface antigens of *E. histolytica*.

A cDNA expression library to study gene expression and control, and to obtain recombinant antigens for immunological studies has been prepared. Restriction fragments generated from a hypervariable region of *E. histolytica* ribosomal DNA have been shown to vary among clones of the same amebal strain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00097-32 LPD

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Physiological and Cytochemical Pathology of Parasitic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.I. Mercado	Research Physiologist	LPD, NIAID
Others:	H.M. Fales	Chief, Laboratory of Chemistry	LPD, CH, NHLBI

COOPERATING UNITS *(if any)*
Waters Life Science Applications Laboratory, Fairfax, VA. (M.P. Strickler)
Department of Chemistry, Georgetown University, Washington, DC. (G. Hammer)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Physiology and Biochemistry

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on the purification of a lytic, anti-trypanosomal factor from the bacterial species, *Pseudomonas fluorescens*, were continued. Reversed-phase high performance liquid chromatography (HPLC) was used in our attempts to obtain fractions suitable for NMR and mass spectroscopic analyses. Although a considerably purified compound was obtained, structural analysis was complicated by the occurrence of Tris in the purified fractions. This chemical was present in the defined medium used for the cultivation of the bacterium and persisted through various chromatographic column elutions. After decontamination of the samples employing several procedures for Tris removal, NMR analysis disclosed the occurrence of glycosidic linkages in this antibiotic but further decontamination will be necessary to obtain a complete structural characterization. The determination of chemical structure is essential before studies on synthesis are initiated and pharmacological and toxicological properties are examined employing *Trypanosoma cruzi* infections.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00098-34 LPD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical mechanisms of energy metabolism in mammalian and parasitic organisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	E.C. Weinbach	Section Head	LPD, NIAID
Others:	L. Diamond	Research Zoologist	LPD, NIAID
	L. Levenbook	Research Chemist	LPB, NIDDK

COOPERATING UNITS (if any)

None

LAB/BRANCH Laboratory of Parasitic Diseases

SECTION Physiology and Biochemistry

INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐x (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Continued studies of the uptake and binding of antidepressant tricyclic drugs (imipramine and chlorimipramine) showed that the binding of chlorimipramine was competitively inhibited by imipramine and its analogs. Kinetic analyses of the equilibrium data revealed that binding of both drugs is saturable, and Scatchard plots indicated a single class of binding sites. The effect of pH on binding affinities showed increased sensitivity of the parasites with increasing pH values, probably owing to altered protonation of the peripheral membrane proteins, and the high pK value (8.3) of the drugs.

Collaborative studies, initiated with Dr. Diamond this year, of the respiratory metabolism of Entamoeba species revealed a specific stimulatory effect of Mn^{++}. Distinct differences in molecular taxonomy also were found among these species.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00099-20 LPD

PERIOD COVERED
October 1989 - September 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biophysical Parasitology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. A. Dvorak	Res. Microbiologist	LPD
Others:	J. P. McDaniel	Biologist	LPD, NIAID
	J.C. Engel	Fogarty Fellow	LPD, NIAID
	T. Nozaki	Fogarty Fellow	LPD, NIAID
	S. Banks	Senior Staff Fellow	OSD, NIAID
	C. P. Mudd	Senior Engineer	ACE, BEIB

COOPERATING UNITS (if any)

Biomedical Engineering and Instrumentation Branch, DRS; Office of the Scientific Director, NIAID; Laboratory of Applied Studies, DCRT

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Physiology and Biochemistry

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is concerned with (a) analyses of the genetic diversity of *Trypanosoma cruzi* and its implications to the epidemiology, course and diagnosis of Chagas' disease, (b) the development of high resolution flow cytometry instrumentation for biomedical research and (c) the utilization of flow cytometry and low light level video microscopy for the analyses of infectious agents. We have established that inter-developmental stage differences exist in both total DNA and kinetoplast and/or nuclear base ratios in *T. cruzi*. Hybridization analyses with DNA probes indicate that *T. cruzi* exhibits restriction fragment length polymorphism (RFLP) and preliminary data indicate this may be due, in part, to amplified DNA or multiple repeated sequences. Although definitely a low frequency occurrence, variants of *T. cruzi* can arise spontaneously which may explain both the appearance and maintenance of *T. cruzi* diversity in nature. Environmental stress can result in the appearance of new and stable variants within cloned stocks.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00102-16 LPD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Disease Caused by Infection with Intracellular Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	F. A. Neva	Chief	LPD, NIAID
Others:	D. Sacks	Research Microbiologist	LPD, NIAID
	C. King	NRSA Fellow	LPD, NIAID
	A. Cooper	Visiting Fellow	LPD, NIAID

COOPERATING UNITS (if any) Ministry of Health, Tegucigalpa, Honduras (C. Ponce); Inst. de Salubridady Enfermadades Trop., Mexico City (O. Velasco); Dept. Biol, Youngstown U, Ohio (R. Kreutzer); Yale U Sch of Pub. Hlth, Dept. of Epidemiology, New Haven, CT (D. McMahon-Pratt); ER Squibb and Sons, Inc., Princeton, NJ (AL Lentnek and R. Williams).

LAB/BRANCH Laboratory of Parasitic Diseases

SECTION Cell Biology and Immunology Section

INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project continues to focus primarily upon the different clinical forms of leishmanial infection in humans, their immune response to leishmanial antigens, and characteristics of the causative parasites.

Although only four new patients were studied in the Clinical Center during the last year, considerable use has been made of peripheral mononuclear cells recovered from them and from previous patients that are obtained by leukophoresis and stored frozen. Dr. Cooper has used such cells for investigating antigen expression on infected cells and Dr. Karp has been developing in-vitro assays for several different lymphokines.

Studies of the typical cutaneous leishmaniasis caused by L. donovani chagasi have continued in Honduras. A survey by Dr. Donce of delayed skin test reactivity in people without history or evidence of clinical disease from an endemic area has disclosed a high rate of positive reactors. Also maximal DTH reactions were found to an antigen prepared from homologous species of organism. We hope to initiate studies on patients with mucocutaneous leishmaniasis.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00108-19 LPD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Immunobiology of Malaria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L.H. Miller	Head, Malaria Section	LPD, NIAID
	S. Kumar	Visiting Associate	LPD, NIAID
Others:	T. McCutchan	Senior Scientist	LPD, NIAID
	Antoniana Krettli	Guest Worker	LPD, NIAID
	Peter Perlmann	Fogarty Scholar	LPD, NIAID
	Hedvig Perlmann	Guest Worker	LPD, NIAID

COOPERATING UNITS (if any)

W. Weiss, Naval Med. Res. Inst.; J.A. Berzofsky, Sr. Scientist, Metabolism Branch, NCI; R. Houghten, M. So, and F. Heffron, Scripps CLinic, LaJolla, CA; M.F. Good, QIMR, Brisbane, Australia; Aiba Agas, U. of Miami; William E. Collins, CDC, Atlanta

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Immunity to asexual erythrocytic parasites in certain rodent malarias is dependent on cell mediated mechanisms that are dependent on CD4+ T cells and the spleen and are independent of antibody. Such immunity can be induced by Salmonella and malarial antigens. The present studies are designed to identify those proteins and T cell epitopes that will lead to protection. In addition, we are developing studies to test constructs in Salmonella that will lead to protection against *Plasmodium falciparum* in *Aotus* monkeys.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00161-13 LPD

PERIOD COVERED October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunochemistry of Parasitic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T.E. Nash	Medical Officer	LPD, NIAID
Others:	F.A. Neva	Chief	LPD, NIAID
	M. Ballard	Medical Staff Fellow	LPD, NIAID
	A.W. Cheever	Assistant Chief	LPD, NIAID
	M.M. Mowatt	Staff Fellow	LPD, NIAID
	B. Gottstein	Visiting Scientist	LPD, NIAID

See below

COOPERATING UNITS (if any)

M.M. Levine, D.A. Herrington, G.A. Losonsky, University of Maryland

LAB/BRANCH Laboratory of Parasitic Diseases

SECTION Host-Parasite Relations

INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
3.8	2.3	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(From above)

S. Banks, D. Alling	Office of Scientific Director	NIAID
P. Pimenta		LPD, NIAID
G. Harriman, W.S. Strober		LCI, NIAID

The variant surface antigen repertoires and the rates these antigens are expressed differ among *Giardia* isolates. In cloned populations trophozoites with new variant antigens are detected every 12-13 generations in isolate WB and once every 6.5 generations in isolate GS. The ability to express variant antigens is genetically determined since some isolates lack genes encoding the surface antigens of other isolates. The variant surface antigens also differ in their ability to resist digestion with the intestinal enzymes trypsin and chymotrypsin. After exposure of sensitive clones to protease, some trophozoites remain alive and these are stably resistant to these enzymes. These antigens may be important for survival of *Giardia* in the intestines.

Neonatal mice infected with *Giardia lamblia* undergo antigenic variation. Immunological studies using this model reveal T cell responses to *Giardia* are limited to the intestine and are not present to the variant antigen.

The need for host immune responses in the successful treatment of cestode infections with praziquantel was studied in *T. taeniaformis* infections in the mice. Humoral factors, e.g., antibody are required for successful therapy in *T. taeniaformis* and probably *T. solium* infections as well.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00162-14 LPD

PERIOD COVERED October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Cytology of Host-Parasite Interactions in Parasitic Protozoa

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D.M. Dwyer	Supervisory Microbiologist	LPD, NIAID
Others:	D.J. Mallinson	Visiting Fellow, FIC	LPD, NIAID
	V.H. Mann	IRTA Fellow	LPD, NIAID
	C. McCarthy-Burke	Biologist	LPD, NIAID

COOPERATING UNITS (if any) Dept.Zool.,Univ.Glasgow(P.A. Bates); Inst.Med.Parasit.,Univ.Bonn (I. Bonsch-Hermes);Depts.Biochem.and Microbiol.,Univ.Victoria (R.W.Olafson, T.W.Pearson & D.L.Tolson); Dept.Biochem.,Univ. Kentucky (S. Turco); Dept.Biol.,Technion Israel Inst. Tech. (D.Zilberstein).

LAB/BRANCH Laboratory of Parasitic Diseases

SECTION Immunology & Cell Biology

INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.83	3.0	0.83

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The cell biology of *Leishmania* is investigated as a model of both intra- and extracellular parasitism. Emphasis is placed on characterizing the biochemical and physiological functions of its surface membrane (SM) and secreted components toward defining their roles in parasite survival and development.

Leishmania promastigotes possess a number of SM transport systems for the active accumulation of certain essential amino acids and nucleosides. In order to identify these, a series of unique, radiolabeled, substrate specific, affinity-binding reagents were devised, synthesized and used to covalently label the SM of living *L. donovani* promastigotes *in situ*. Such labeling was specifically and competitively inhibited (>90+%) in the presence of excess unlabeled substrate. Cumulatively, two different, externally oriented, SM transporters were identified in these studies. The first, specific for methionine was composed of 2 polypeptides with apparent molecular weights of ~54 and 56 kDa and the second was specific for adenosine transport and consisted of a single polypeptide chain of ~33 kDa. Studies are in progress to further characterize these essential proteins. Additional chemical properties of the SM acid phosphatase were delineated. The secretory acid phosphatase (SAcP) was shown to possess the unique $[PO_4\text{-}6Gal\beta1,4Man]_n$ repetitive epitope in common with the SM lipophophoglycan (LPG). Both SAcP and LPG were terminally co-processed in and transported through the Golgi compartment. Restriction and genetic analyses of wild type and LPG- mutants are being used to identify genes involed in LPG expression. A 420 bp DNA coding fragment for SAcP was cloned and used to identify and isolate a full length copy of this gene. Methods were devised for the continuous *in vitro* cultivation of "amastigote-like" forms of *L. donovani* at 37°C. The availability of such forms should facilitate studies of parasite gene regulated differentiation and development.

The current results are of relevance toward the development of improved diagnostic, chemotherapeutic and immunoprophylactic agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00197-11 LPD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunoregulation and immune recognition in filariasis and non-filarial diseases.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T.B. Nutman	Senior Investigator	LPD, NIAID
Others:	E.A. Ottesen	Senior Investigator	LPD, NIAID
	C.L. King	Medical Staff Fellow	LPD, NIAID
	A. Limaye	Howard Hughes Research Scholar	LPD, NIAID
	A. Klion	Medical Staff Fellow	LPD, NIAID

COOPERATING UNITS (if any)

Department of Allergy and Clinical Immunology, Univ. of Montreal, Montreal, Canada (G. Delespesse); Dept. of Onchocerciaisis, SNEM, Guatemala City, Guatemala (G. Zea-Flores), Univ. National de Benin (Dr. Massouboudgi), Anna Univ., Madras India (Dr. K. Jayaraman)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Clinical Parasitology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to delineate the mechanisms involved in regulating the humoral and cellular responses in patients with filariaisis and other disease states. Immunoregulatory studies have examined the phenomenon of antigen-specific anergy in microfilaremic patients by showing this anergy to be a result of tolerance (by clonal deletion) rather than active suppression.

In vitro models of parasite-antigen driven antibody production as well as parasite-specific and HTLV-I transformed T cell clones have been developed to understand in more detail those mechanisms regulating antibody production (particularly IgG and IgE) in filarial and non-filarial diseases. Recombinant lymphokines and neutralizing antibodies to them have provided additional tools for defining the mediators involved in this regulation.

Qualitative analysis of filaria-specific IgE and IgG in loiasis, lymphatic filariasis, and onchocerciasis have indicated patterns of antigen recognition which differ among groups of patients with different clinical manifestations of filariasis. Using these techniques, possible vaccine targets have been identified for use in onchocerciasis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00208-10 LPD

PERIOD COVERED October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Isolaton and Characterization of Plasmodial Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. F. McCutchan	Microbiologist	LPD, NIAID
Others:	G. McConkey	IRTA Fellow	LPD, NIAID
	S. Gigliotti		LPD, NIAID
	W. Write	Guest Worker	LPD, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH Laboratory of Parasitic Diseases

SECTION Malaria Section

INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have been characterizing the ribosomal RNA of different malarial parasites. Information from these studies has important ramifications for the development of malaria diagnostics, the understanding of evolutionary relationships among the different malarial species and the understanding of control of the parasites developmental cycle.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00240-09 LPD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigenic Analysis of Sexual Stages of Malaria Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Kaslow	Medical Staff Fellow	LPD, NIAID
	L.H. Miller	Head, Malaria Section	LPD, NIAID
Others:	I. Quakyi	Guest Worker	LPD, NIAID
	D. Rawlings	Guest Worker	LPD, NIAID
	D. Keister	Biologist	LPD, NIAID
	J. Coligan	Branch Chief	BRB, NIAID
	K. Williamson	Guest Worker	LPD, NIAID

COOPERATING UNITS (if any) Hazelton Labs, Vienna VA; Cetus Corp, Emeryville CA (J Ninberg); Scripps Clin Res Fnd, La Jolla CA (J Ninberg); Scripps Clin Res Fnd, La Jolla CA (R. Houghton); QIMR Brisbane Australia (M. Good); Wyeth Laboratories Inc., Radnor PA(P. Hung), Chiron Corp., Ameryville CA (P. Barr)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 10892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.6	3.2	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have set our goals on, first, developing a 25 kDa sexual stage surface antigen, Pfs25, as a potential vaccine candidate; second, cloning the genes for the other known target antigens of transmission blocking immunity; third, identifying new target antigens on sexual stage parasites; and finally, understanding the molecular mechanisms involved in fertilization of malarial parasites. We have also begun to study the role of parasite glucose-6-phosphate dehydrogenase (G6PD) and its role in the putative protection afforded by G6PD deficiency in humans.

Previously we had cloned the gene encoding Pfs25, a prime candidate antigen for a transmission blocking vaccine. Pfs25 has now been expressed in bacteria, yeast, vaccinia-infected mammalian cells and transiently transfected COS cells. Data from mice immunized with vaccinia-produced Pfs25 are very encouraging: sera from mice either inoculated with live, recombinant vaccinia or immunized with membrane extracts of mammalian cells infected with recombinant vaccinia block transmission of malaria. To begin further developing Pfs25 as a vaccine candidate, we have pursued engaging a number of commercial biotechnology companies in Cooperative Research and Development Agreements (CRADA). Cloning the other target antigens has been a problem. New approaches, such as mammalian expression systems, are being explored in addition to pursuing strategies previously successful, such as protein microsequencing and screening bacterial expression libraries. A microgamete (male) specific monoclonal antibody has been developed and may provide the entre' we need to understand the molecular mechanisms involved in fertilization in the malaria parasite.

Finally, we have demonstrated that the malaria parasite expresses G6PD constitutively, and independently of the G6PD status of the host. In our attempt to clone the parasite G6PD gene by complementation in *E. coli*, we have cloned the gene encoding a related enzyme, glucose phosphate isomerase (PGI).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00241-09 LPD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Identification of Receptors for Merozoite Invasion of Erythrocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.H. Miller	Section Head	LPD, NIAID
Others:	J. Adams	Staff Fellow	LPD, NIAID
	D. Hudson	Microbiologist	LPD, NIAID
	T. Wellems	Sr. Staff Fellow	LPD, NIAID
	D. Kaslow	Staff Fellow	LPD, NIAID
	X. Fang	Visiting Fellow	LPD, NIAID
	G. Ward	Visiting Associate	LPD, NIAID

COOPERATING UNITS *(if any)*

WRAIR, Washington, DC (F. Klotz and J.D. Haynes); Hazelton Laboratories, Vienna, VA (J. Rener);Case Western Reserve Univ., Cleveland, OH (M. Aikawa)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.3	4.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The merozoite interacts in a receptor specific manner with the erythrocyte surface and is the stage upon which immunity may work to block invasion. Thus, merozoite surface components are of interest because of their role in erythrocyte recognition and as antigens for induction of protective immunity. We are identifying *P. knowlesi*, *P. vivax* and *P. falciparum* receptors for attachment to monkey and human erythrocytes. To understand the molecular basis for this variation we are studying one antigen on the merozoite surface that undergoes antigenic variation. The components in the junction and the signaling after merozoites make contact with erythrocytes is also under study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00244-09 LPD

PERIOD COVERED October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Developmental Adaptation of *Trypanosoma cruzi* to the Vertebrate Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Sher	Section Head	LPD, NIAID
Others:	S. Heath	Visiting Fellow	LPD, NIAID

COOPERATING UNITS (if any)

Yale University (K. Joiner, G. Ogden)
University of Sao Paulo, Brazil (T. L. Kipnis, D. V. Tambourgi)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this project we have been studying developmental adaptations of *T. cruzi* to the vertebrate host and, in particular, the molecular basis of adaptive changes occurring during the differentiation of epimastigotes (vector stage) to metacyclic trypomastigotes (infective stage).

This year we succeeded in cloning a previously characterized developmentally regulated molecule from the parasite which has decay accelerating factor (DAF) activity inhibiting parasite mediated complement activation. The molecule has extensive sequence homology with human DAF.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00246-08 LPD

PERIOD COVERED October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular Studies of the Genome and Surface of *Schistosoma mansoni*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Sher	Section Head	LPD, NIAID
Others:	E. J. Pearce	Visiting Associate	LPD, NIAID
	P. Brindley	Special Volunteer	LPD, NIAID
	C. Champion	Special Volunteer	LPD, NIAID

COOPERATING UNITS (if any) Biomedical Research Institute, Rockville, MD (F. Lewis); (R. Houghten); Fiocruz, Belo Horizonte, Brazil (R. Correa-Oliveira, L. Viera.) Molecular Vaccines Inc., Gaithersburg, MD. (V. de la Cruz)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Immunology and Cell Biology Section

INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS: 3.1	PROFESSIONAL: 2.6	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this project is to characterize schistosome surface molecules relevant to immunity, to analyze the genome of the parasite in relation to specific biological properties of the organism and to clone important schistosome immunogens.

A. Development of a paramyosin BCG recombinant. cDNA's encoding paramyosin were cloned into BCG for vacome testing.

B. Genetic analysis of natural schistosome populations. Using a rDNA probe, the genetic hetergeneity and geographic variation of a collection of *S. mansoni* isolates from Brazil was characterized.

C. Creation of an expression clone bank for T epitope screening. A battery of 500 clones from a *S. mansoni* expression cDNA were selected for use in identifying important antigens recognized by T cells from vaccinated animals.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00248-09 LPD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetics and Physiology of Vector Capacity in Anopheline Mosquitoes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.W. Gwadz	Senior Scientist	LPD, NIAID
	L.H. Miller	Section Head	LPD, NIAID
	R.K. Sakai	Expert	LPD, NIAID

D.C. Kaslow, Med Staff Fellow, LPD, NIAID
C. Graziosi, Guest Worker, LPD, NIAID
P.A.J. Romans, Visiting Fellow, LPD, NIAID
H. G. Coon, Senior Investigator, LG, NCI
K.-P. Sieber, Staff Fellow, LPD, NIAID
K. Mizuuchi, Senior Investigator, LMB, NIDDK
M. Huber, Visiting Fellow, LPD, NIAID
M.S. Lee, Guest Researcher, LMB, NIDDK

COOPERATING UNITS (if any) Dept of Entomology, U of MD, Col Prk, MD (Dr. M Ma); Inst of Parasitology, U of Rome (Dr. M Coluzzi); Inst of Pathology, Case Western Reserve Sch of Med (Dr. M Aikawa); Ecole Nat de Med, Bamako, Mali (Dr. YT Toure); U of MD, Biotechnology Ins (Dr. R. Colewell).

LAB/BRANCH Laboratory of Parasitic Diseases

SECTION Malaria Section

INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.2	5.2	2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The molecular biology of anopheline mosquitoes is being studied in relation to the capacity of these vectors to transmit malaria. Systems for cloning and transposing genes into mosquito germ lines are being developed. Factors which render mosquitoes refractory to malarial infection, with special emphasis on mechanisms which encapsulate developing parasites, are being defined. Linkage of refractory genes to genes regulating blood-meal associated physiological events, e.g., vitellogenin synthesis, will be used to enhance refractory responses.

Additional parasiticidal mechanisms from within and outside of mosquitoes are being sought with special emphasis on the evaluation of magainins, cecropins and defensins, their physiological effects on in vivo parasite development and the molecular biology of the genes coding for their production.

The ability to identify, clone and transpose genes which regulate refractory mechanisms should permit the development of mosquito lines incapable of transmitting malaria for use in malaria control schemes.

PHS 6040 (Rev. 1/84)

GPO 814-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00251-09 LPD

PERIOD COVERED October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Immunologic Studies on Schistosomiasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Sher	Section Head	LPD, NIAID
Others:	E. J. Pearce	Visiting Associate	LPD, NIAID
	S. L. James	Program Officer	MIDP, IRP, NIAID
	A. W. Cheever	Assistant Chief	LPD, NIAID
	J. M. Grzych	Special Volunteer	LPD, NIAID

COOPERATING UNITS (if any)

DNAX Research Institute, Palo Alto, CA (R. Coffman)
University of Alberta (T. Mosmann)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Immunology and Cell Biology Section

INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.0	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this project is to study mechanisms of immunity, immune evasion and immunopathology in schistosomiasis with the ultimate goals of developing an experimental vaccine suitable for human trials as well as understanding the pathogenesis of disease.

In work completed this year, the schistosome egg was shown to be the major stimulus of the Th2 response observed in infected mice. In addition, eggs as well as oviposition were shown to down regulate Th1 cytokine (IFN-γ, and IL-2) production in vaccinated mice as well as mice with prepatent infections. IL-10 (CSIF) a newly discovered cytokine was found to be produced by T cells from infected mice and its activity may explain the observed down-regulation in Th1 cytokine synthesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00253-09 LPD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Immunological Responses to Filarial Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.A. Ottesen	Senior Investigator	LPD, NIAID
Others:	T.B. Nutman	Medical Officer	LPD, NIAID
	C. King	Medical Staff Fellow	LPD, NIAID
	A. Klion	Medical Staff Fellow	LPD, NIAID
	N. Raghavan	Fogarty Fellow	LPD, NIAID
	E. Lobos	Fogarty Fellow	LPD, NIAID

(See below)

COOPERATING UNITS (if any) Indian Cncl of Med Res, Madras, India (S Tripathy, V Kumaraswami); Anna Univ, Madras, India (K Jayaraman); Dept of Hlth, Guatemala (G Zea-Flores); CPqAM/FIOCRUZ, Recife, Brazil (G. Dreyer, A. Coutinho); U. of Minnesota (E Holland); U. of Alabama (D.O. Freedman); Centers for Dis. Control (R.B. Lal)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	1.8	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(See above)	S. Mahanti	NRSA Fellow	LPD, NIAID
	A. Limaye	Howard Hughes Fellow	LPD, NIAID
	F.A. Neva	Chief	LPD, NIAID
	H. Francis	Senior Investigator	OSD, NIAID
	R.B. Nussenblatt	Clinical Director	NEI
	C.C. Chan	Senior Investigator	NEI

The purpose of this project is to define the humoral and cellular immune responses that relate to immunodiagnosis, immunopathology and protective immunity in patients with lymphatic filariasis, onchocerciasis, and loiasis.

Serologic assays for filarial infection based on IgG4 antibody detection have greatly enhanced specificity because the IgG4 subclass is 'restricted' from recognizing epitopes such as phosphocholine that are common constituents of parasitic and other infectious organisms. Such IgG4 responses have been shown to take 6-9 months to develop despite strong immune stimulation. A diagnosistic test for new or pre-patent onchocerciasis infections has been developed using a purified recombinant 16 kD protein (OV-16).

Histopathologic and serologic evidence implicates the eosinophil and its granule proteins as a primary determinant of the posttreatment reactions seen in onchocerciasis and lymphatic filariasis; IL-5 appears to be the primary determinant of eosinophil responses.

Populations with bancroftian filariasis or onchocerciasis have been examined to define immunologic parameters that distinguish "naturally immune" from infected individuals. A 43kD protein from infective larvae that may be a protective immunogen has been cloned from a *W. bancrofti* expression library, and the gene is being sequenced. Similar studies in onchocerciasis have also identified differentially recognized molecules, that are being purified.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00255-09 LPD

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies of the Immunologic Responses to Non-Filarial Parasitic Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.A. Ottesen	Senior Investigator	LPD, NIAID
Others:	L. Andrade	Visiting Scientist	LPD, NIAID
	T.B. Nutman	Medical Officer	LPD, NIAID

COOPERATING UNITS (if any)
Aga Khan Mecical Center, Karachi, Pakistan (R. Hussain)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host Parasite Relations

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.4	0.3

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Serial serum specimens collected 15 years ago from untreated patients with acute schistosomiasis mansoni have been previously studied for the evolution of their IgG and IgE antibody responses to different schistosome antigens. Because of the recently recognized importance of IgG4 antibodies both for diagnostic specificity and for blocking allergic reactions to the parasite, this unique collection of sera has been restudied to define the kinetics of a developing specific IgG4 antibody response. Interestingly, it appears that while antibodies of IgG1, IgG2 and IgG3 subclasses develop within weeks of acute infection, specific IgG4 antibodies (to both egg and adult schistosome antigen) do not develop for 6-9 months after infection. This piece of information is of particular interest, since there are almost no other instances where the natural development of an IgG4 response in humans has been recorded.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00256-09 LPD

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Developmental Biology of Leishmania Promastigotes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Sacks	Senior Investigator	LPD/NIAID
Other:	T. Brodin	Guest Researcher	LPD/NIAID
	P. Pimenta	Guest Researcher	NCI

COOPERATING UNITS (if any)

Dr. Sam Turco, Dept. of Biochemistry, University of Kentucky

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During growth within the sand fly and within axenic culture, *Leishmania* promastigotes undergo differentiation from a non-infective to an infective or metacyclic stage which is uniquely adapted for life in the vertebrate. For some species of *Leishmania* (*L. donovani* and *L. major*) this development is accompanied by a substantial modification of the surface lipophosphoglycan (LPG) which is the major surface glycoconjugate of these cells. During metacyclogenesis, the LPG is developmentally modified such that it no longer binds the lectin PNA, it expresses a novel carbohydrate epitope, and the number of phosphorylated saccharide units expressed increases 2-3 fold. Immunogold labeling of the surface LPG revealed a 10nm thickening in the LPG coat, which assumed a filamentous appearance on freeze-fracture preparations. The LPG on Old World promastigotes forms a densely packed surface coat which effectively masks other surface molecules, such as GP63. We have presented evidence that the elongation of the LPG serves to promote the complement resistance of inoculated metacyclics by preventing insertion of the membrane attack complex into the membrane. LPG does not appear to be expressed on the surface of *L. major* or *L. donovani* amastigotes, suggesting that the surface coat is a stage specific' structure which undoubtedly influences the survival and behaviour of the parasite within the alimentary tract of the sand fly. These vector biological studies have only recently been initiated. LPG has also been investigated on New World species, and while metacyclic promastigotes of these species express saccharide units which are altered in composition, there is no elongation of the LPG, and this is consistent with the continued serum susceptibility of these infective forms.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00257-09 LPD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunology of Strongyloidiasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	F.A. Neva	Chief	LPD, NIAID
Others:	P.J. Brindley	Special Volunteer	LPD, NIAID
	T. Fleischer	Chief, Clin., Immun. Service	CPD, Clin. Center
	C. Brown	Medical Staff Fellow	LIR, NIAID
	W. Blattner	Chief, Viral Epid., Section	EEB, NCI
	S. Greenberg	Medical Staff Fellow	MB, NCI

(See below)

COOPERATING UNITS (if any)

U. of Calif. at S.F., Dept. Pathology (J. McKerrow); SEMA, Inc., Rockville (T. Moskal), U. of the West Indies, Jamaica (Prof. Morgan, Ralph Robinson and S. Terry); Project SIDA, Kinshasa, Zaire (J. Perriens); Georgetown U. contract (W.T. London).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	0.8	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(From above)

E. Ottesen, T. Nash, T. Nutman Senior Investigators LPD, NIAID

This project involves both clinical studies of humans infected with the intestinal nematode, *Strongyloides stercoralis*, and analysis of parasite antigens and immunologic responses to them. The parasite is of particular interest because of its unusual biologic properties and the fact that it can produce serious, even fatal, disease in certain immunosuppressive states.

Several approaches to affinity purification of the parasite larval protease were attempted. One method was conventional use of monoclonal antibody, and the other involved conjugating one of the zinc metallo-protease inhibitors to sepharose and elution of the protease from such a substrate. The evidence for purification of the protease by either method remains equivocal. A continuing problem in interpretation of results is the variability in molecular size of the protease under different physico chemical conditions.

Clinical and serologic studies have continued for evidence of strongyloidiasis in different population groups with high prevalence of infection with the retroviruses, HIV and HTLV-1. The possibility that serum IgE response may be an important factor in controlling strongyloides infection is also being examined. The parasite did not appear to be important as an opportunistic infection in a limited number of AIDS patients in Zaire, and their IgE responses were not impaired. In contrast, mean serum IgE levels were reduced in certain groups of patients positive for HTLV-1 in Jamaica.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00347-08 LPD

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Schistosomal Hepatic Fibrosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. W. Cheever, Head, Host-Parasite Relations Section, LPD, NIAID

Others:	J. Macedonia	Bio. Lab. Tech., LPD, NIAID
	Y. Xu	Visiting Fellow, LPD, NIAID
	A. Sher	Head, Immunology, LPD, NIAID
	J. M. Grzych	Visiting FEllow, LPD, NIAID
	P. Scott	Senior Staff Fellow, LPD, NIAID

COOPERATING UNITS (if any)

Laboratoire de Pathologie Cellulaire du Foie, Institut Pasteur, Lyon (Jean-Alexis Grimaud).
Department of Medicine, USUHS (Fred Finkelman).

LAB/BRANCH Laboratory of Parasitic Diseases

SECTION Host-Parasite Relations Section

INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hepatic fibrosis and the granulomatous response to eggs of schistosome species pathogenic for man are studied in mice in relation to parasitologic parameters of infection. Treatment of mice with anti-IL-5 monoclonal antibody depleted S. japonicum infected mice of eosinophils. Granulomas without eosinophils were only slightly reduced in size and had fibrosis equivalent to that in untreated mice. Splenic lymphocytes from S. japonicum infected animals secrete IL-5 but not interferon τ after stimulation with egg antigen or ConA. These findings parallel the reactions examined previously in S. mansoni infected mice and suggest the importance of Th-2 cells, but not IL-5, for the formation of granulomas and the development of fibrosis in murine S. japonicum infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00350-08 LPD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

DNA Analysis of Parasites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.E. Nash	Medical Officer	LPD, NIAID
Others:	R. Adam	Assistant Professor, Medicine	University of Arizona
	M. Mowatt	Staff Fellow	LPD, NIAID
	B.Y. Nguyen	Medical Staff Fellow	LPD, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Analysis of two variant surface antigens from two different isolates continue. Both are cysteine-rich, contain cys-x-x-cys motifs, are highly transcribed, and are found only in the clone expressing the variant. A 170 kD cysteine-rich protein (CRP) has a repeating subunit which was found to contain the epitope which reacts with Mab 6E7. Genes have been identified which can be used to differentiate isolates. One (C4) is highly transcribed in non-WB-like *Giardia* and is not found in WB-like *Giardia*. The function of C4 is not known.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00439-06 LPD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Therapeutic Studies in Human Filariasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	E.A. Ottesen	Senior Investigator	LPD, NIAID
Others:	T.B. Nutman	Medical Officer	LPD, NIAID
	C. King	Medical Staff Fellow	LPD, NIAID
	A. Klion	Medical Staff Fellow	LPD, NIAID

COOPERATING UNITS (if any) Indian Council of Medical Research, Madras, India; (R. Prabhakar and V. Kumaraswami); Madras Medical College, Madras, India (V. Vijayasekaran); Peace Corps Medical Office (M. Mulligan); Centro de Pesquisas Aggeu Magalhaes, Recife, Brazil (A. Coutinho, G. Dreyer); Dept of Health, Cotonou, Benin (J.P. Manshande).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.8	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Ivermectin has been shown in trials in South India to be effective in clearing microfilaremia in patients with bancroftian filariasis. A placebo-controlled trial of ivermectin and the currently used drug, diethylcarbamazine (DEC), has shown equivalent efficacy for ivermectin and DEC for 3 months, but by 6 months the ~18-20% microfilarial recurrence rate in the ivermectin-treated patients was significantly greater than the ~6% recurrence rate in the DEC group. Side effects were essentially identical for both groups. Thus, though ivermectin appeared somewhat less effective than DEC at 6 months, its single-oral-dose mode of administration and toxicity no greater than that of DEC means that this drug should engender greater patient compliance and, therefore, have the potential to be much more effective in mass-treatment filariasis control programs than DEC. Current trials in South India and Brazil (coordinated with trials elsewhere in the world) indicate that very low doses of ivermectin (10 mcg/kg) clear microfilariae with even fewer side reactions and, when followed by a second higher dose, may lead to extremely prolonged microfilarial clearance.

Loiasis acquired by expatriate visitors to endemic areas has been characterized in earlier studies at NIH as showing marked clinical and immunologic hyperresponsiveness to the filarial parasite. The hypothesis that this hyperresponsive syndrome contrasted with a hyporesponsive ('tolerized') state, both clinical and immunologic that appeared in those infected individuals native to the endemic regions was proven by clinical and laboratory studies in Benin, West Africa.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00483-05 LPD

PERIOD COVERED

October 1989 to September 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetics of Drug Resistance and Red Cell Invasion in Malaria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas E. Wellems	Sr. Staff Fellow	LPD, NIAID
Others:	David Peterson	Research Fellow	LPD, NIAID
	Stephen Dolan	Med. Staff Fellow	LPD, NIAID
	Annie Walker-Jonah	Fogarty Fellow	LPD, NIAID
	Kathleen Creedon	Guest Worker	LPD, NIAID

COOPERATING UNITS *(if any)*

Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. (Wilbur K. Milhous); Washington University, St. Louis, MO (Donald J. Krogstad, Ilya Gluzman); Catholic University, Washington, D.C. (Pradip Rathod)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our investigations are directed at the mechanisms of drug resistance and red blood cell invasion by the malaria parasite *Plasmodium falciparum*. Genetic crosses, linkage analysis, and differential screening methods are being employed to characterize genes involved in these mechanisms. Resistance to two common antimalarial drugs, pyrimethamine and proguanil, has been found to result from point mutations in the *P. falciparum* dihydrofolate reductase gene. Different point mutations produce different responses to each drug, explaining why strains refractory to one drug may still respond to the other. Chloroquine resistance has been examined in a cross of two *P. falciparum* clones. Examination of 16 independent recombinant progeny from the cross indicates that chloroquine resistance is controlled by a single gene or by a closely linked group of genes. Linkage analysis studies are being performed to search for the locus controlling chloroquine resistance. Alternative pathways of red cell invasion are activated in some parasite clones when sialic acid residues are cleaved from the red cell surface by neuraminidase treatment. Differential and subtractive screening methods are being used to clone genes involved in these pathways.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00487-04 LPD

PERIOD COVERED October 1, 1989 - September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies on the Quantitative Parasitology of Schistosome Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. W. Cheever, Chief, Host-Parasite Relations, LPD, NIAID

Others: J. Macedonia Bio. Lab. Tech., LPD, NIAID

COOPERATING UNITS (if any)

Laboratory of Statistical Methodology, DCRT (J. Mosimann)

LAB/BRANCH Laboratory of Parasitic Diseases

SECTION Host-Parasite Relations Section

INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Present efforts are concentrated on evaluating the role of host reactions on parasitologic parameters (see Z01 AI 00347) and on the development of models for the kinetics of egg laying, egg destruction and egg excretion in *S. mansoni* and *S. japonicum* infections. Accurate assessment of the rate of egg destruction is important for these models. Eggs in the tissues of *S. japonicum* infected mice persisted in unchanged numbers in the tissues for at least 1 year after chemotherapeutic cure of infections of 8 weeks duration. The destruction of *S. mansoni* eggs in murine tissues may also be slower than previously thought.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00494-04 LPD

PERIOD COVERED October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Analysis of T Cell Responses in Human Leishmaniassis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D.Sacks	Senior Investigator	LPD,NIAID
Others:	A. Cooper	Fogarty Fellow	LPD,NIAID
	C. Karp	Guest Researcher	
	F. Neva	Chief	LPD,NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Cell Biology and Immunology Section

INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL: 2.8	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Immunity to infection by the intracellular parasite *Leishmania* is mediated by sensitized T cells, however, the antigens which they recognize have not been defined nor has direct recognition of infected macrophages by T cells been clearly demonstrated. We have previously shown that the cell-mediated response of individuals with healed or healing forms of cutaneous leishmaniasis occurs to as many as 50-70 distinct antigens, however, it is unclear whether all the antigens capable of inducing a T cell response are important in protective immunity. Our hypothesis is that endogenous antigens derived from living amastigotes and presented during the early stages of infection are limited, and that these antigens are most involved in protection. We therefore tried to establish whether infected human monocytes could present antigens to autologous T cells, and we compared the responses of *Leishmania* specific T cells to antigen-pulsed monocytes and to infected monocytes. Using populations of human monocytes 95-100% infected with *L. donovani* amastigotes, we demonstrated the ability of infected monocytes to present endogenously derived antigen. T cells from cutaneous patients have been further separated into CD4 negative, CD8 negative or gamma delta negative populations, and their responses compared. CD4- cells from old world cutaneous patients lost their response to both infected and antigen pulsed monocytes. New World cutaneous patients responded better to infected monocytes and demonstrated little reduction in proliferative response after CD4 depletion, and the cytokine responses (gIFN and GMCFS) could be resonstituted by addition of IL-2. Thus CD8 cells seem to be activated in some patients and not others, and their activation may be more dependent on antigens produced by living parasites.

The antigens recognized by immune T cells on infected cells are being identified by generating T cell lines and clones reactive with autologoulsly infected cells and using these cells to screen fusion proteins selected from cDNA libraries with Kala-azar serum. It is our hope that this system will serve as a model for studying the cell biological aspects of proccessing and presentation of antigens derived from intracellular parasites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00512-03 LPD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Definition Of Filarial Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T. B. Nutman	Senior Investigator
Others:	N. Raghavan	Visiting Scientist
	Edgar Lobos	Visiting Scientist
	Amy Klion	Medical Staff Fellow
	E. A. Ottesen	Senior Investigator

COOPERATING UNITS (if any)

New England Biolabs, Beverly, Mass (F. Perler, L. McReynolds, C. Maina), Department of Geographic Medicine, University of Alabama Birmingham (D. Freedman)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Clinical Parasitology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.6	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In the study of the human filariases, progress has been hampered by 1) the lack of defined parasite antigens; 2) the broad immunological cross-reactivity seen among the eight filarial species of humans; and 3) the dearth of abundant parasite material. The objectives of this project are to define and generate filarial proteins that are important in inducing parasite-specific immune responses in the human host and to understand, at a molecular level, the differences among related filarial species.

cDNA and genomic libraries have been either constructed (*Brugia malayi*, *Loa loa* and *Wuchereria bancrofti*, *Onchocerca volvulus*) or made available (*Onchocerca volvulus, Brugia malayi* larval cDNA library) so that screening with defined patient sera, patient T cells or parasite DNA could be performed. Recombinant antigens and probes have been identified that a) induce T cell responses in an antigen-specific manner; b) may be in part protective in onchocerciasis; c) can distinguish among related filarial species by restriction fragment length polymorphisms, PCR or direct Southern blotting; d) encode immunoreactive and potentially protective molecules of *W. bancrofti*; e) identify repeated segments of either the *W. bancrofti* genome or that of *Loa loa*; and f) may be of potential diagnostic importance.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AI 00579-01 LPD

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies on Immune Regulation in Toxoplasmosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Sher	Senior Investigator	LPD, NIAID
Others:	R. Gazzinelli	Special Volunteer	LPD, NIAID
	F. Hakim	Staff Fellow	I, NCI
	G.M. Shearer	Senior Investigator	I, NCI

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Parasitic Diseases

SECTION: Immunology and Cell Biology Section

INSTITUTE AND LOCATION: NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.6	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The overall aim of this project is to analyze the immune response to *Toxoplasma gondii* in order to define which cellular immune components and parasite target antigens are involved in the control of infection and its breakdown in immunocompromised hosts.

In this year's work, protective immunity in a murine vaccine model was analyzed and shown to require a synergistic interaction between $CD4^+$ and $CD8^+$ cells as well as the production of IFN-γ.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00517-03 LPD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ligand-receptor Interactions in Parasite Attachment to Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Keith Joiner	Senior Investigator	LPD, NIAID
Others:	Steve Fuhrman	Medical Staff Fellow	LPD, NIAID
	G. Furtado	Guest Researcher	LPD, NIAID
	J. Schweinle	IPA	LPD, NIAID
	G. Ogden	Staff Fellow	LPD, NIAID

COOPERATING UNITS (if any)

A. Tenner, American Red Cross; A. Ezekowitz, Children's Hospital, Harvard Univ.; H. Kleinman, LSBA, NIDR; Y. Yamada, LSBA, NIDR; H. Krivan, LSB, NIADDK

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Unit of Microbial Pathogenesis

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.1	3.0	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Terminated

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00535-03 LPD

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of Intracellular Compartments Containing Microorganisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Keith Joiner	Senior Investigator	LPD, NIAID
Others:	B.F. Hall	Medical Staff Fellow	LPD, NIAID
	J.E. Schweinle	IPA	LPD, NIAID
	D. Rotrosen	Medical Staff Fellow	LCI, NIAID
	A.B. Tartanian	Technician	LPD, NIAID
	Steve Fuhrman	Medical Staff Fellow	LPD, NIAID

COOPERATING UNITS *(if any)*
Ira Mellman, Associate Professor, Yale Univ.; Lloyd Kasper, Associate Professor, Dartmouth College; John Albert, Technician, Georgetown Univ.; Tomas Ganz, Associate Professor, UCLA

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Unit of Microbial Pathogenesis

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.7	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Terminated

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00536-03 LPD

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies on Transcriptional Regulation in Trypanosoma cruzi

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Keith Joiner	Senior Investigator	LPD, NIAID
Others:	G.B. Ogden	Staff Fellow	LPD, NIAID
	B.F. Hall	Medical Staff Fellow	LPD, NIAID
	G. C. Furtado	Guest Researcher	LPD, NIAID

COOPERATING UNITS (if any)

none

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Unit of Microbial Pathogenesis

INSTITUTE AND LOCATION
NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Terminated

PHS 6040 (Rev. 1/84) GPO 914-918

LABORATORY OF VIRAL DISEASES
1990 ANNUAL REPORT
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LABORATORY OF VIRAL DISEASES
ANNUAL REPORT, 1990
SUMMARY

The Laboratory of Viral Diseases carries out investigations on the molecular biology of viruses, the interactions of viruses with host cells, the pathogenesis of viral diseases, and host defense mechanisms. These studies are designed to increase fundamental knowledge as well as to facilitate the development of new approaches to the prevention and treatment of virus infections. Current topics of basic research include virus entry into cells, regulation of gene expression, mechanisms of DNA replication, assembly and transport of viral proteins and particles, action of virus growth factors, determinants of virus virulence, host resistance genes, and viral targets of humoral and cellular immunity. Applied areas of investigation include the development of recombinant expression vectors, candidate vaccines, and antivral agents. These studies involve a wide range of DNA and RNA viruses including human immunodeficiency virus.

REGULATION OF VIRUS GENE EXPRESSION

DNA viruses provide advantageous systems for studying basic aspects of gene expression and can be used as expression vectors. Several different virus systems are currently under investigation by members of LVD.

Poxviruses. Poxviruses are large DNA viruses that replicate in the cytoplasm of infected cells. In order to express and replicate their genomes outside of the nucleus, poxviruses must encode enzymes for these processes. These feature make poxviruses, among which vaccinia virus is the prototype, unique for studying the regulation of transcription. Additional information regarding transcription also is needed to improve vaccinia virus expression vectors, which are currently being evaluated as live vaccines against several diseases including AIDS. During the past year, progress has been made in identifying viral genes including RNA polymerase subunits and early and late transcription factors. Evidence was obtained for a cascade mechanism of gene regulation.

Vaccinia virus encoded RNA polymerase. The DNA-dependent RNA polymerase of vaccinia virus contains 8 to 10 polypeptides. Previous studies from this laboratory indicated that the RNA polymerase subunits are virus-encoded and the genes for one large and one small subunit were identified. The gene encoding an additional small subunit, rpo18, has now been found. Transcription of rpo18 occurs early in infection and the untranslated leader contains a 5' poly(A) sequence that is probably generated by a 3' to 5' slippage mechanism. The role and mode of formation of this leader is under investigation.

(Ahn and Moss)

Vaccinia virus early transcription factor. The early transcription factor (VETF) is a heterodimer consisting of 70-kD and 82-kD subunits. The genes encoding both subunits were discovered and the smaller one has sequence motifs in common with a superfamily of DNA and RNA helicases. The transcription factor subunits are expressed late in infection, just prior to their packaging in virus particles.

(Gershon and Moss)

Three transactivators of late gene expression. Transfection methods were used to identify three vaccinia virus genes with zinc finger motifs that encode transactivators of late gene expression. The data support a regulatory cascade in which the parental viral genome serves as a template for RNA polymerase and early transcription factors that are packaged within the virus particle whereas newly replicated DNA is accessible to intermediate and late transactivators.

(Keck, Baldick and Moss)

Vaccinia virus capping enzyme. The genes predicted to encode the vaccinia virus capping enzyme were co-expressed in Escherichia coli and an active heterodimeric enzyme was produced. Recombinant capping enzyme should be a useful reagent for labeling RNA and enhancing RNA translatability in vitro.

(Guo and Moss)

Parvoviruses. Parvoviruses are small viruses that contain a single-stranded DNA genome. One well studied member of this family, adeno-associated virus (AAV), is dependent for replication on coinfection with an adenovirus or herpesvirus. AAV gene expression appears to depend on cellular and helper virus products.

Regulation of expression of AAV structural proteins by alternative splicing and initiation codon usage. Previous data suggsting that structural protein B is initiated from an ACG intitiation codon has been confirmed by site directed mutagenesis. The latter procedure also was used to determine the derivation of structural protein A from an alternately spliced transcript. All 3 structural proteins are required for infectivity, since mutants that fail to produce any one of these three are not infectious.

(Sebring, Wong, Muralidahar and Rose)

VIRUS DNA REPLICATION

Viruses are useful systems for analyzing the diversity of mechanisms employed in DNA replication. In addition, the virus encoded factors provide potential targets for chemotherapy.

Poxviruses. Poxviruses provide a unique experimental system, since the enzymes and factors are encoded within the virus genome and replication occurs in the cytoplasm. Previous studies revealed that vaccinia virus DNA is replicated as concatemeric structures which are resolved into unit length genomes with hairpin ends.

Vaccinia virus DNA concatemer junctions are processed by conservative strand exchange. Analysis of a series of symmetrical deletion mutations demonstrated that the presence of a DNA sequence ATTTAGTGTCTAGAAAAAAA on both sides of the apical segment of the concatemer junction is crucial for resolution. The orientation of each sequence with respect to each other and to the axis of symmetry is also of critical importance. A model incorporating site-specific recombination and oriented branch migration was proposed to account for resolution of the vaccinia concatemer junction.

(Merchlinsky)

Drug-resistance domains of vaccinia virus DNA polymerase. Previous work established that substitution of methionine for leucine at residue 645 of DNA polymerase confers resistance to the drug aphidicolin. This is a highly conserved part of DNA polymerase and efforts are being made to determine the importance of this region by site directed mutagenesis.

(DeFilippes)

Herpes simplex virus (HSV). HSV and other members of the herpesvirus family are significant human pathogens. The study of HSV DNA synthesis is useful as a model for eukaryotic DNA replication and for designing new anti-viral strategies. Previous work from this laboratory demonstrated that seven HSV genes are necessary and sufficient for authentic origin-dependent DNA replication.

Functional and physical interactions between the HSV-1 DNA polymerase and the product of the UL42 gene. The DNA polymerases from HSV infected mammalian cells, and from insect cells infected with recombinant baculoviruses expressing products of UL30 (catalytic subunit) and UL42, were purified as heterodimers of 181 kD. The complex is more highly processive and has a 10-fold higher binding affinity for linear duplex DNA than the catalytic subunit alone.

(Gottlieb and Challberg)

Characterization of the HSV primase/helicase complex. The products of three HSV genes, UL5, UL8, and UL52, from HSV-infected or recombinant baculovirus-infected cells form a complex with both helicase and primase activities. Translocation of the helicase is in the 5' to 3' direction and requires nucleoside triphosphate (preferably ATP) hydrolysis. The primer formed is homogeneous in size and more than 95% is 8 nucleotides long.

(Sherman and Challberg)

Adeno-associated virus. The AAV genome is a linear single-stranded DNA molecule with partially palindromic inverted terminal repeats. Replication of AAV DNA occurs by a mechanism involving self-primed synthesis.

Role of non-structural proteins. In restrictive monkey cells, non-structural proteins are not made but AAV DNA synthesis occurs. Instead of normal length molecules, long concatemers accumulate suggesting that non-structural proteins are required for resolution.

(Sebring, Wong, Muralidhar and Rose)

SYNTHESIS, ASSEMBLY, AND TRANSPORT OF VIRAL PROTEINS AND PARTICLES

Information regarding the folding, assembly, and transport of viral glycoproteins and the formation of virus particles is of intrinsic interest and practical importance, since it dermines the antigenic structure recognized by neutralizing antibody.

Influenza virus. The trimeric influenza hemagglutinin (HA) molecule is the best characterized viral envelope protein and therefore serves as an excellent model. Additionally, influenza virus remains a significant human pathogen.

Site of trimerization of the influenza HA. Evidence that trimerization occurs in a post endoplasmic reticulum (ER) compartment was obtained by use of conformation-specific monoclonal antibodies. This concept was supported by genetic engineering experiments in which an ER retention signal was added to the carboxy terminus of the HA leading to accumulation of the HA as a monomer.

(Eisenlohr, Russ, Yewdell and Bennink)

Compartmentalization of the ER in the presence of brefeldin A (BFA). BFA is a fungal metabolite that interferes with the trafficking of vesicles between the ER and Golgi complex. By following the localization of monomeric and trimeric forms of HA, evidence was obtained for a distinct and previously unrecognized subcompartment of the ER.

(Russ, Eisenlohr, Yewdell and Bennink)

Human immunodeficiency virus (HIV). The HIV envelope protein is the best target of neutralizing antibody and is therefore of particular importance.

Oligomeric structure of the HIV envelope glycoprotein. The envelope glycoprotein of HIV-1, HIV-2, and SIV are synthesized as monomers and assembled into olgomers prior to cleavage into gp120 and gp41 subunits or transport to the plasma membrane. When the envelope proteins of HIV-1, HIV-2, and SIV are expressed in the same cell, heterodimers are formed indicating a functionally conserved domain involved in subunit-subunit recognition.

(Earl, Doms, Chakrabarti and Moss)

VIRUS-HOST INTERACTIONS

HIV envelope protein/human CD4 mediated fusion. HIV infects cells by binding to surface CD4 molecules and directly fusing with the cell membrane. The preparation of recombinant vaccinia viruses that express CD4 or HIV-1 envelope protein has permitted the study of fusion in a wide variety of human and non-human cells. In all cases examined, fusion occurred when the HIV envelope protein was expressed in non-human cells and CD4 was expressed in human cells. The reverse, however, occurred rarely suggesting the lack of a helper or presence of an inhibitory factor in non-human cell membranes.

(Ashorn, Berger and Moss)

Soluble CD4 promotes specific dissociation of gp120/gp41 complex. Fusion between HIV and cell membranes is thought to involve structural changes in the envelope protein molecule following the binding to CD4. Addition of soluble CD4 to cells expressing the envelope glycoprotein was shown to promote specific dissociation of gp120. Preliminary studies suggest that a region of CD4 distinct from the gp120 binding site is involved in this effect.

(Broder and Berger)

Fusion of vaccinia virus with the cell membrane. There are two infectious forms of vaccinia virus: the INV form, isolated by disrupting infected cells, and the EEV form, present in the medium. A lipid mixing assay, based on dilution of a fluorescent probe, was used

to establish that both forms of virus fuse with plasma membranes in a pH independent manner. Nevertheless, a monoclonal antibody that prevented fusion of INV had no effect on fusion of EEV suggesting that different viral proteins may be involved in entry of the two forms of virus.

(Doms and Moss)

Inhibition of the complement cascade by the major secretory protein of vaccinia virus. A 35 kD secretory protein encoded by vaccinia virus, with homology to the family of complement regulatory proteins, was shown to bind to complement component C4b and to inhibit the classical complement cascade in vitro. Mutant virues that fail to make this protein are unable to synthesize a secreted complement inhibitor. These data suggest that the 35 kD protein serves as a defenese molecule to help the virus evade host immune responses.

(Kotwal, Isaacs and Moss)

Regulation of the host inflammatory response by a the product of a cowpox virus gene. The sites of infection in the chicken chorioallantoic membrane produced by certain cowpox mutants are heavily infiltrated with heterophils and macrophages. This enhanced inflammatory effect can be produced by specific mutation of the gene encoding a 38 kD protein with homology to the serpin family. These data are consistent with a model in which the 38 kD protein directly or indirectly inhibts the generation of chemotactic molecules or blocks the interaction of such molecules with cells mediating the inflammatory response.

(Palumbo and Buller)

Deletion of the vaccinia virus ribonucleotide reductase gene affects virulence. Vaccinia virus encodes both subunits of ribonucleotide reductase which presumably catalyzes the formation of deoxynucleotides. Mutagenesis studies indicate that the ribonucleotide reductase genes are not required for growth of vaccinia virus in tissue culture but are needed for full virulence when mice are inoculated intracranially.

(Palumbo and Buller)

VIRAL IMMUNOLOGY

Cytotoxic T cells (CTLs) play an important role in eradicating intracellular pathogens and cancer cells. It is well established that for recognition by CTL, processed antigens must associate with MHC molecules, apparently by binding to a groove in the latter.

Processing of antigens and association with MHC class I molecules. Brefeldin A (BFA), a specific inhibitor of exocytosis, was found to inhbit the presentation to MHC class I molecules of endogenously synthesized proteins and proteins derived from input non-infectious virions but not of exogenously added peptides. These data suggested that protein antigens are processed via the cytosolic route prior to their egress from the trans-Golgi complex. Further evidence that the association of antigen with class I molecules occurs in the ER was obtained by using recombinant vaccinia virus expressing the adenovirus E19 protein to retain MHC molecules in the ER.

(Cox, Yewdell, Eisenlohr and Bennink)

HIV-1 CTL epitopes. An epitope within HIV-1 reverse transcriptase was found to be recognized by both mouse and human CD8+ CTL. This broad specificity suggests that it might be appropriate for inclusion in a vaccine. A peptide, corresponding to an immunodominant epitope of the HIV-1 gp120 that is recognized by class I MHC restricted CTL, was shown to also induce CD4 T cell help for itself. The processing of enodgenously synthesized gp160 for recognition and lysis by CD4+ T cells required that the protein remain attached to the luminal/extracellular membrane face by a hydrophobic anchor sequence.

(Earl and Moss)

VIRUS EXPRESSION VECTORS

Use of expression vectors has become an important part of recombinant DNA technology. Vaccinia virus is now widely used for expression of proteins in mammalian cells and has proven particularly useful for determining the targets of humoral and cellular immunity. Recombinant vaccinia virus is currently being tested as a candidate AIDS vaccine in humans and a rabies vaccine in wild-life. The unique DNA integration and other properties of some parvoviruses suggest that they might be of particular use for gene therapy (see section on antiviral agents).

Transient dominant selection of recombinant vaccinia viruses. A new general method of selecting recombinant vaccinia viruses was developed. The gpt antibiotic resistance marker is not retained in the final recombinant virus and hence this procedure can be used serially to introduce several foreign genes.

(Falkner and Moss)

Attenuation of recombinant vaccinia viruses expressing interleukin-2 (IL-2). Previous studies showed that recombinant vaccinia viruses expressing IL-2 are attenuated in athymic nude mice. Such recombinant vaccinia viruses have now been shown to be attenuated in normal primates. Despite this attenuation, no significant reduction in immunogenicity was noted.

(Flexner and Moss)

CHARACTERIZATION OF NEWLY DISCOVERED VIRUSES

Human herpesviruses. Until recently, only 5 human herpesviruses were known. A few years ago, human herpesvirus 6 (HHV-6) was discovered and this was followed by the isolation of human herpesvirus 7 (HHV-7) in this laboratory. Both of these new human herpesviruses are lymphotropic. HHV-6 has been associated with roseola, a disease of infants.

Isolation of HHV-7. HHV-7 was isolated from CD4+ T cells of healthy individuals. The virus was most likely induced from a latent state during in vitro cultivation. Electron microscopy revealed a typical herpesvirus structure. The genome is approximately 145,000 bp long and hybridization indicated some relatedness to HHV-6. Nevertheless, HHV-6 and HHV-7 are immunologically distinct.

(Frenkel, Katsafanos, Schirmer and Wyatt)

Epidemiology of HHV-7. Immunofluorescence (IFA), immunodot, and Western blot analyses have been developed to investigate the prevalence of HHV-7 in the population. Using the IFA test, 93% of 27 individuals were seropositive for both HHV-6 and HHV-7. Further studies indicate that seroconversion usually occurs in childhood. DNA probes are being developed for additional investigations.

(Wyatt and Frenkel)

Biology of HHV-6 and HHV-7. Studies on the assembly and maturation of HHV-6 indicate the existence of an intranuclear compartment where capsids acquire their tegument prior to transport into the cytoplasm. The tegumented capsids attach to and bud into cytoplasmic vacuoles, thus acquiring the virion envelope. T-cell activation is required for HHV-6 replication, however replication is delayed by high IL-2 concentrations.

(Roffman, Katsafanos, Schirmer and Frenkel)

DEVELOPMENT OF ANTIVIRAL AGENTS

HIV

CD4-Pseudomonas exotoxin hybrid protein (CD4-PE40). A genetically engineered hybrid toxin, composed of the HIV envelope protein-binding domains of CD4 and the translocation and ADP ribosylase domains of Pseudomonas exotoxin A, has been constructed. Previous data indicated that the toxin was specific for HIV-infected cells. These results have now been extended to HIV-infected peripheral blood lymphocytes and macrophages. In addition, CD4-PE has been shown to act synergistically with AZT and DDI, inhibitors of reverse transcriptase, and in combination with these drugs can eliminate HIV from infected cultures.

(Ashorn, Moss and Berger)

HIV protease inhibitors. Protease inhbitors synthesized by Upjohn Co. have been shown to block cleavage of gag and gag-pol precursor proteins. As a result, immature particles with unprocessed gag proteins are released from cells.

(Ashorn, Karacostas and Moss)

HIV antisense RNA parvovirus vectors. AAV vectors that express RNA complementary to the 5' end of HIV transcripts were constructed. Cell lines containing integrated vectors inhibted expression of genes under control of promoter and enhancer sequences within the HIV long terminal repeat by 95% or more. Furthermore, HIV replication in such cells was inhibited to a similar degree.

(Chaterjee, Rose and Wong)

POXVIRUSES

Interference of poxvirus replication by inhibitors of arachidonic acid metabolism. Several inhibitors of arachidonic acid metablolism have been found to specifically interfere with the replication of orthopoxviruses.

(Palumbo and Buller)

ADMINISTRATIVE CHANGES

During the past year, Dr. Mark Challberg was appointed Head of the Macromolecular Biology Section. A Molecular Genetics Section with Dr. Niza Frenkel as Head and a Viral Immunology Section with Dr. Jack Bennink as Acting Head were established. As usual there has been a healthy turnover of young investigators who came to NIAID to further their research training. Some have gone on to academic appointments and others to positions in biotechnology.

Honors, awards, and service. Members of LVD received numerous invitations to give major lectures and seminars in the United States as well as in foreign countries. Drs. Challberg, Moss, and Rose serve on the editorial boards of one or more scientific journals.
Dr. Challberg is an advisor to the National Science Foundation and Dr. Moss is a member of the National Academy of Sciences.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00123-24-LVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Replication of Poxvirus DNA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Merchlinsky	Staff Fellow	LVD, NIAID
Others:	B. Moss	Chief	LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Poxviruses provide a unique system for studying the synthesis of DNA. Required enzymes and factors are encoded within the viral genome and DNA synthesis and processing occurs within the cytoplasmic compartment of the cell. Therefore, it has been possible to apply genetic and biochemical approaches to the study of DNA replication. My effort has been towards ascertaining the structure and mode of replication of the poxvirus genome with particular emphasis placed on understanding the processing of the telomere-like hairpin structure and the enymes involved in its replication. The replication of vaccinia virus proceeds through concatemeric intermediates which are resolved into unit length DNA. Plasmids containing the telomere replicative intermediate were, after transfection into cells infected with vaccinia virus, replicated and resolved into linear minichromosomes with sealed terminal hairpins. This provides a system to study the *cis* acting DNA sequences required for telomere resolution. The introduction of a series of symmetrical insertions, deletions, and site-directed oligonucleotide mutations has demonstrated that a DNA sequence, highly conserved among poxviruses, as well as the palindromic structure of the concatemer junction, is essential for resolution, and that resolution occurred by conservative strand exchange. A model for resolution involving site-specific recombination and orientated branch migration is consistent with this data.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00126-17 LVD

PERIOD COVERED October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional Analyses of Vaccinia DNA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Frank M. DeFilippes Research Physicist LVD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1	PROFESSIONAL: 1	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A mutation in the vaccinia virus DNA polymerase gene which changes amino acid residue 645 in the polymerase from leucine to methionine results in a vaccinia virus mutant which is resistant to aphidicolin (AP). Residue 645 is in a region of conserved amino acids in the carboxyl portion of the polymerase. I use site directed mutagenesis to change the conserved amino acids near residue 645 to determine how essential these amino acids are for enzyme activity. The strategy is to use the marker transfer procedure to introduce linked alterations one of which alters a codon for a conserved residue while the other confers AP resistance. Virus plaque formation in the presence of AP indicates that the polymerase can function with the new amino acid since recombination between the linked changes should be minimal. To test the method, I used an altered DNA with a neutral change from valine to alanine, at residue 645. Marker transfer produced AP-resistant plaques whose progeny contained DNA with the codon for alanine at the proper position. However, when I tried to introduce drastic changes, such as replacing asparagine (residue 640) with aspartic acid, or serine (residue 641) with proline, or tyrosine (residue 643) with aspartic acid, the attempts failed. Although AP-resistant plaques were formed after marker transfer, the progeny from the plaques contained only the wild-type codon at the proper position. The reason for this result is not obvious, so the experiment will be improved and repeated. One improvement removes contaminating DNA in the polymerase chain reaction (PCR) which amplifies the altered DNa. Another advance involves the ability to extract DNA from the virus so that the DNA is suitable for amplification without undue manipulation. Finally, a method was developed which allows rapid sequencing of DNA from the PCR.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00294-09 LVD

PERIOD COVERED October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Structure and Function of Adenovirus DNA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. A. Rose	Section Head	LVD, NIAID
Others:	E. Sebring	Research Chemist	LVD, NIAID

COOPERATING UNITS (If any)
R. McPherson, M.D., Department of Pathology, Georgetown University Hospital, Washington, DC; D. Klessing, Waksman Institute, Piscataway, NJ.

LAB/BRANCH Laboratory of Viral Diseases

SECTION Molecular Structure Section

INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)
- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Among objectives of these studies has been the application of physical, biochemical and biological techniques to characterize the structure and function of certain sequences (e.g., the inverted terminal repeats) and genes of adenovirus (Ad) DNA. In earlier studies, we first identified and characterized the VA RNA gene/transcript and inverted terminal repeats in Ad DNA. We have continued to investigate the specific functions of several early Ad genes, e.g., the VA and DNA-binding protein genes. Our results indicate that these latter genes are involved in the regulation of translation of certain viral mRNAs. Among methods used are gradient sedimentation, DNA cleavage with restriction endonucleases, gel electrophoresis, base sequence analysis, and DNA transfection.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00295-09-LVD

PERIOD COVERED October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Helper Factors Required for Expression of the Adeno-Associated Virus Genome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. A. Rose	Section Head	LVD, NIAID

COOPERATING UNITS (if any)

L. Mishra, Senior Staff Fellow, Division of Blood and Blood Products, CBER, FDA

LAB/BRANCH Laboratory of Viral Diseases

SECTION Molecular Structure Section

INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The main objectives of this project are (i) to identify the helper virus genes that regulate expression of defective human parvovirus (AAV) genomes, and (ii) to define their respective roles in AAV replication. We previously mapped the adenovirus genes required for AAV replication and continue to investigate their specific helper functions. Similar studies are in progress with herpes simplex viruses. Among methods used are specific immunofluorescence, cleavage of DNA with restriction endonucleases, DNA cloning, gel electrophoresis, blot-hybridization analyses, and DNA transfection of both simian and human cell lines.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00296-09-LVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization and Production of Parvovirus Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. A. Rose	Section Head	LVD, NIAID
Others:	E. Sebring	Research Chemist	LVD, NIAID
	K. Wong	Medical Staff Fellow	LVD, NIAID
	S. Muralidahar	Visiting Fellow	LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The main objectives of these studies are (1) to identify and characterize all proteins that are specified by the defective human parvovirus (AAV) and to determine similarities and differences with autonomous parvovirus proteins, (2) to define the mechanism(s) by which the AAV proteins arise, and (3) to define specific functions of the AAV proteins. We have identified at least four AAV non-structural proteins. At least one of these proteins is necessary for viral DNA replication. Post-translational processing does not account for production of any AAV structural proteins although they share large segments of sequences-in-common. It is now clear, however, that these proteins originate from independent in-frame initiations. Mechanisms that regulate expression of AAV proteins are of fundamental interest, and we have shown that one AAV structural protein is initiated by a codon (ACG) not known previously to act as an initiation codon in higher eukaryotes. Furthermore, our current findings (i) support a "scanning mechanism" in the translational expression of polycistronic eukaryotic mRNAs, and (ii) demonstrate that alternative mRNA splicing is required for effective translational expression of the largest AAV capsid protein. Among methods used are site-directed mutagenesis, affinity chromatography, gel electrophoresis, in vitro translation of viral RNA, DNA transfection, immunoprecipitation, and Western blotting.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00297-09-LVD

PERIOD COVERED October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism and Regulation of Adeno-associated Virus DNA Replication

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. A. Rose	Section Head	LVD, NIAID
Others:	E. Sebring	Research Chemist	LVD, NIAID
	S. Chatterjee	Visiting Associate	LVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH Laboratory of Viral Diseases

SECTION Molecular Structure Section

INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.65	0.65	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects X☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary objective of this project is to define molecular and biochemical mechanisms involved in eukaryotic DNA synthesis. To approach this problem, we are investigating adeno-associated virus (AAV) DNA replication in both *in vivo* and *in vitro* systems. We have shown that AAV DNA synthesis can be initiated *in vitro* in cell-free extracts and that replicating forms that correspond to those identified *in vivo* can be synthesized with either endogenous or exogenously added templates. Recently, we have shown that AAV "rep" proteins may not be required for the synthesis of AAV concatemeric DNA intermediates, but that one on more of these proteins is required to resolve the long intermediates into unit length duplexes. The observed mode of AAV DNA replication may serve as a model for telomere replication in eukaryotes. Among methods used are affinity chromatography, gel electrophoresis, DNA sequence analysis, and restriction cleavage of DNA molecules.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00298-09-LVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Vaccinia Virus as an Expression Vector

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Moss	Chief	LVD, NIAID
Others:	O. Elroy-Stein	Special Volunteer	LVD, NIAID

COOPERATING UNITS *(if any)*

B. Murphy, LID, NIAID; T. Fuerst, Molecular Vaccines; F. Chisari, Scripps Institute

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☐ (a1) Minors
 ☐ (a2) Interviews

☐ (b) Human tissues

☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Vaccinia virus has been developed into a eukaryotic expression vector. A chimeric gene is formed by ligating vaccinia virus transcriptional regulatory signals to a foreign protein coding sequence. Homologous recombination is used to insert the chimeric gene into a non-essential region of the vaccinia virus genome. Several different methods have been developed to select or screen the recombinant vaccinia viruses. The recombinant viruses can then be used to express proteins in a variety of tissue culture cells or animals. A novel modification of the recombinant vaccinia virus expression system which utilizes the bacteriophage T7 RNA polymerase and T7 transcriptional regulatory signals was developed. The prokaryotic RNA made in mammalian cells by this system, however, was not efficiently capped. Since the 5' cap structure is important for stability and translatability, this presented a severe problem. Stability was achieved by using DNA encoding the 5' end of a T7 transcript with potential to form a stem-loop structure. Translatability was obtained by employing the untranslated leader sequence of encephalomyocarditis virus, which confers cap-independent ribosome binding. With the newly modified system, after 24 hours of infection the recombinant protein comprises approximately 10% of the total cell protein. Several new plasmid victors that facilitate and increase the expression of genes were developed. In addition, a new method of selecting recombinant vaccinia viruses that allows the insertion of several genes was introduced.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00306-09-LVD

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Orthopoxvirus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. M. L. Buller	Expert	LVD, NIAID
Others:	C. Duarte	Bio. Lab. Tech. (Micro)	LVD, NIAID
	G. Palumbo	IRTA Fellow	LVD, NIAID
	W. Chen	Fogarty Fellow	LVD, NIAID

COOPERATING UNITS (if any)

T. Frederickson, U. of Connecticut; D. Pickup, Duke University; L. McIntyre, Vector Lab; J. Sechler, LCI, NIAID; D. Hruby and S. Child, Oregon State U; T. Eling, NIEHS.

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION: NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.4	2.4	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this project, we focused our studies on the genetic basis both of orthopoxvirus virulence and of host resistance to virus infection. The acquired knowledge should contribute toward development of safe, effective recombinant vaccinia virus vaccines for animal and human use. A deletion mutant of cowpox virus has been constructed which lacks a functional gene for a 38 kDa protein involved in the generation of a hemorrhagic pock on the chorioallantoic membrane of the chicken embryo. The results to date suggest that the 38 kDa protein directly or indirectly inhibits the generation of chemotactic molecules which are elicited during virus replication in the CAM or, alternatively, blocks the interaction of these molecules with cells of the inflammatory response. Preliminary data suggested that the lipoxygenase pathway of arachidonic acid metabolism may be the target of the 38 kDa protein. The use of various inhibitors of arachidonic acid catabolism have indicated that this fatty acid is required for orthopoxvirus replication and may be necessary directly or indirectly for the assembly of virus specific-membranes. A second orthopoxvirus mutant, which lacked a functional gene encoding ribonucleotide reductase, was shown to be mildly attenuated when injected into BALB/c mice by the intracranial route of inoculation. *In vitro* and *in vivo* models are being developed to examine the virus-encoded functions which are important for replication in the epidermis and dermis, and enable the virus to spread from the local site of inoculation.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00307-09-LVD

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Regulation of Vaccinia Virus Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B. Moss	Laboratory Chief	LVD, NIAID
Others:	B. -Y. Ahn	Visiting Associate	LVD, NIAID
	B. Amegadzie	Visiting Fellow	LVD, NIAID
	C. J. Baldick	Pre-IRTA	LVD, NIAID
	P. Gershon	Visiting Associate	LVD, NIAID
	J. Keck	NRC Fellow	LVD, NIAID
	N. Harris	Visiting Fellow	LVD, NIAID
	H. Yuwen	Visiting Fellow	LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Viral Diseases

SECTION: Genetic Engineering Section

INSTITUTE AND LOCATION: NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.7	7.2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Vaccinia virus has a genome of about 185,000 base pairs that encodes approximately 200 polypeptides. These genes are expressed within the cytoplasm in a coordinated fashion, so that some polypeptides are made before, and others after, DNA replication. Enzymes and factors needed for early transcription are packaged within the infectious particle, while those needed for late transcription are present in the cytoplasm of infected cells. The aim of this project is to determine the mechanisms regulating gene expression.

Progress in understanding early and late transcription has been made this past year. Several viral genes required for transcription were identified. These include an RNA polymerase subunit of 18,000 daltons, both subunits of the early transcription factor VETF, and three transactivators of late gene expression. Evidence was obtained for a cascade mechanism of gene regulation in which the early transcription factors are made late during the previous infection cycle and packaged in virions, the intermediate factors are made early in infection, and the late factors are made at intermediate times. The DNA present in the infecting virus particle serves as a template for transcription of early genes but naked or newly replicated DNA is required as a template for intermediate and late genes.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00416-07-LVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Recombinant Vaccines against Retroviruses Associated with Leukemia and AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B. Moss	Laboratory Chief	LVD, NIAID
Others:	S. Chakrabarti	Visiting Associate	LVD, NIAID
	P. Earl	Microbiologist	LVD, NIAID
	V. Karacostas	Special Volunteer	LVD, NIAID
	R. Padmanabhan	IPA	LVD, NIAID

COOPERATING UNITS (if any)

LIR, NIAID, LI, NIAID; Metabolism Branch, NCI, LTCB, NCI; LCMS, NCI; Massachusetts General Hospital, Immuno AG; Molecular Vaccines, Johns Hopkins, UpJohh Co.

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.3	4.3	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human immunodeficiency virus (HIV) is the etiological agent of acquired immunodeficiency syndrome (AIDS). At present there is no effective vaccine against this disease and therapeutic agents provide only limited help. The objects of this project are to characterize HIV antigens, determine the targets of humoral and cell-mediated immunity, and to use this information to develop candidate vaccines. We have constructed recombinant vaccinia viruses containing HIV genetic information. These viruses have been used as live experimental vaccines to immunize animals, to synthesize HIV proteins in tissue culture, to make targets for cytotoxic T cells, and study CD4-envelope protein interactions. Removal of cryptic poxvirus transcription termination signals that exist within the HIV-1 envelope gene was necessary for optimal expression. The envelope proteins of HIV-1, HIV-2 and SIV were shown to exist in oligomeric forms that might enhance their immunogenicity and binding to the CD4 receptor on lymphocytes. The formation of heteroligomers between HIV-1 and HIV-2 or SIV envelope proteins indicates that the domain is highly conserved. The gag-pol genes of HIV-1 were expressed and the proteins were assembled into particles that budded from the plasma membrane. Such particles represent a novel noninfectious form of HIV that might have potential as a vaccine. In addition, gag particle formation and processing have provided a useful system for testing protease inhibitors as potential therapeutic agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI-00445-06 LVD

PERIOD COVERED

October 1, 1989 to September 30,1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Viral DNA Replication

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. D. Challberg	Section Head	LVD, NIAID
Others:	D. Fierer	Medical Fellow	LVD, NIAID
	J. Gottlieb	IRTA Fellow	LVD, NIAID
	G. Sherman	IRTA Fellow	LVD, NIAID
	D. Klinedinst	IRTA Fellow	LVD, NIAID

COOPERATING UNITS (if any)

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Herpes simplex virus is a useful model for studying the mechanisms involved in DNA replication in eukaryotic cells. Our current efforts are directed toward studying this process with purified proteins. Seven viral genes are both necessary and sufficient to carry out authentic origin-dependent DNA replication. Ongoing biochemical analyses in several laboratories support the idea that the products of these seven genes all participate directly in viral DNA synthesis. We are currently using both biochemical and molecular genetic approaches to understand the function of these polypeptides in detail.

The HSV DNA polymerase purified from infected HeLa cells consists of a stable complex of at least two polypetides: UL30, the catalytic subunit, and UL42. We have purified both subunits free of each other using recombinant baculoviruses. Although the purified catalytic subunit has the same specific activity as the UL30/UL42 complex using activated DNA as template, the complex is much more active on a long single-stranded DNA template primed with a short oligonucleotide. Several lines of evidence support the view UL42 increases the efficiency of the DNA polymerase by increasing its processivity.

The UL9 protein binds specifically to the HSV origins of replication. Determination of the hydrodynamic properties of purified UL9 protein has shown that the protein interacts with itself to form a dimer in solution, and possibly higher order strudctures when bound to the origin of replication. Current experiments on UL9 are aimed at characterizing these higher order structures in more detail. In addition to origin binding, the purified UL9 protein UL9 protein possesses an intrinsic 3' to 5' helicase activity that is capable of unwinding short duplex DNA segments in an ATP-dependent reaction. The role of this unwinding reaction in DNA replication is being investigated.

The UL5, UL8, and UL52 polypeptides form a three protein complex that has both helicase and primase activities. Data from several labs suggest that UL8 has primase acitivity, and UL5 and UL52 acti together as a helicase. We are currently carrying out directed mutagenesis of these proteins to gain further insight into their activities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00538-03 LVD

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Interaction of Human Immunodeficiency Virus with the CD4 Receptor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. Berger	Expert Consultant	LVD, NIAID
Others:	B. Moss	Laboratory Chief	LVD, NIAID
	P. Ashorn	IRTA Fellow	LVD, NIAID
	C. Broder	NRC Associate	LVD, NIAID
	P. Robbins	Spec. Volunteer	LVD, NIAID

COOPERATING UNITS (if any)
I. Pastan, V. Chaudhary, D. Fitzgerald, NCI

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Genetic Engineering Section

INSTITUTE AND LOCATION
NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	2.2	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

CD4 is a surface molecule of human helper T-lymphocytes that serves as the receptor for human immunodeficiency virus (HIV), the causative agent of AIDS. We have focused on two areas related to CD4 and HIV:

1) Design of CD4-based therapeutics for the treatment individuals infected with HIV. We have continued to study a genetically engineered hybrid toxin (CD4-PE40) which is targeted to selectively kill HIV-infected cells. Extending previous findings with continuous human T-cell lines, we have found that the hybrid toxin is active against HIV-infected primary T-cells, as well as against infected cells of the monocyte/macrophage lineage (continuous lines and primary cells). Most importantly, CD4-PE40 shows highly synergistic anti-HIV activity with reverse transcriptase inhibitors (AZT, DDI), in both continuous T-cell lines and primary T-cell cultures. We observed that combination treatment with CD4-PE40 plus AZT can completely eliminate infectious HIV from cultures of continuous T-cell lines.

2) Structure/function studies of the interaction of CD4 with the HIV-1 envelope glycoprotein (gp120/gp41 complex). The focus of this work is to identify structural changes in the envelope glycoprotein which occur upon CD4 binding, and which are involved in virion fusion with the cell membrane. Soluble CD4 was found to promote specific dissociation of gp120 from the envelope glycoprotein complex. Studies with synthetic peptides and soluble CD4 molecules from other species indicate that this dissociation effect may involve a region within the first domain of the CD4 molecule, distinct from the region previously implicated in high affinity binding to gp120. We are developing strategies to determine whether this structural change is associated with exposure of the presumed fusagenic N-terminus of the gp41 molecule.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00539-03-LVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Virus-Cell Interactions, Viral Pathogenesis, and Host Immunity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B. Moss	Laboratory Chief	LVD, NIAID
Others:	S. Isaacs	Medical Staff Fellow	LVD, NIAID
	R. Blasco	Visiting Associate	LVD, NIAID
	Y. Zhang	Visiting Fellow	LVD, NIAID

COOPERATING UNITS (if any)

M. Frank and R. McKenzie, LCI, NIAID
R. W. Doms and R. Bluminthal, NCI

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	3.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A successful virus infection usually involves entry into the cell; uncoating, expression and replication of the genome; assembly and release of infectious virus particles; and defense against specific and non-specific host immune mechanisms. Some genes are required for replication in any cell, whereas others are only important in certain ones. Still other genes have no role in vitro and are advantageous only during animal infection. Studies during the past year have contributed to our understanding of the mechanism of virus entry. Evidence was obtained that both intra- and extracellular forms of vaccinia virus fuse with the cell membrane in a pH independent manner. A large number of genes that are not required for growth in tissue culture have been identified. The conservation of these genes in vaccinia virus and other members of the orthopoxvirus genus signifies that the protein products have an important role in virus-host interactions. We have given the name virokine to one class of such proteins that are secreted from infected cells. The first member of this class is the vaccinia virus growth factor, VGF. We have identified two additional members. One is a 35,000 molecular weight protein that has both structural and functional similarities to the human complement 4b binding protein. The viral protein is able to bind to C4b and to block the classical complement pathway *in vitro* and may provide defense against the host immune system. Another protein of this class has a molecular weight of 14,000. Although the function of the protein of this class has a molecular weight of 14,000. Although the function of the protein is unknown, deletion of the gene attenuates the pathogenicity of vaccinia virus for mice.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AI 00540-03 LVD

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular Biology of Human Herpesviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	N. Frenkel	Supervisory Microbiologist	LVD, NIAID
Others:	E. Schirmer	Biologist	LVD, NIAID
	G. Katsafanas	Biologist	LVD, NIAID
	B. Avidor	Visiting Fellow	LVD, NIAID
	L. Wyatt	Senior Staff Fellow	LVD, NIAID
	R. Danovich	NRC Fellow	LVD, NIAID
	E. Roffman	Visiting Associate	LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Viral Diseases

SECTION

INSTITUTE AND LOCATION: NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.7	4.7	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our studies this past year concerned the lymphotropic human herpesviruses 6 and 7 (HHV-6 and HHV-7). The salient features of these studies can be summarized as follows: 1. We have isolated a new human herpesvirus, HHV-7, from CD4+ T cells purified from peripheral blood lymphocytes (PBL) of a healthy individual. The original RK strain and three additional HHV-7 strains isolated since then were induced from latency in PBL by conditions promoting T cell activation. The virus is distinct from the 6 previously characterized human herpesviruses as revealed by restriction enzyme and blot hybridization analyses. 2. The HHV-7 genome is approximately 145 kb in size. Approximately 43 kb have thus far been cloned. 3. We have developed an indirect immunofluorescence test and Western blot analyses specific for HHV-6 and HHV-7. In a limited survey we have shown that HHV-7 is ubiquitous in humans, in similarity with other human herpesviruses. Seroconversion occurs in childhood albeit at a later age than HHV-6. Studies designed to determine potential association of HHV-7 with human disease(s) are underway. 4. EM examination of HHV-6 and HHV-7 infected cells revealed details in virus egress from the infected cells. This study led to the recognition of specialized intranuclear structures in which the virus appears to acquire its tegument. 5. We have continued our earlier studies showing that T cell activation was required for HHV-6 replication. This work has documented that complete progression in the cell cycle was required for efficient virus replication. We have also shown that IL-2 at high concentrations inhibited HHV-6 replication. The effect may reflect dependence of virus replication on cell cycle and does not appear to be mediated by γ-interferon. 6. We have found that infection of quiescent cells with cell free preparations of HHV-6 resulted in inhibition of T cell activation by mitogen (phytohemagglutinin) or by antigen (tetanus toxoid). Similar immunosuppressive effect was observed following infection of the cells by herpes simplex virus, and appears to depend on

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00541-03-LVD

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Folding Assembly and Transport of Viral Glycoproteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. W. Yewdell	LVD, NIAID
	J. R. Bennink	LVD, NIAID
Others:	G. Russ	LVD, NIAID
	L. Eisenlohr	LVD, NIAID

COOPERATING UNITS *(if any)*
T. Bachi, Institute for Immunology and Virology, Zurich

LAB/BRANCH
Laboratory of Viral Diseases

SECTION

INSTITUTE AND LOCATION
NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.00	1.75	.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The improvement of current antiviral vaccines and the development of novel vaccines depends on improving our understanding of viral attachment and fusion glycoproteins. Critical insight into the structure and function of viral glycoproteins is provided by studying their biosynthesis in virus-infected cells. We have studied the biosynthesis of influenza virus hemagglutinin. The HA is a critical antigen for protecting individuals against influenza, which remains a major cause of morbidity and mortality nationally and internationally. In addition, the fact that the HA is the best characterized viral glycoprotein allows it to serve as a model for elucidating general features of viral glycoprotein structure, function, and antigenicity.

In the past year we have pursued two ongoing studies of HA biosynthesis and transport. First, we have further characterized the site of HA assembly into trimers. Our findings suggests that, contrary to prevailing opinion, the HA trimerizes in a post-endoplasmic reticulum (ER) compartment, probably the early portion of the Golgi complex (GC). Second, we have further characterized the effect of brefeldin A on HA assembly and transport. BFA is a fungal metabolite that interferes with the normal trafficking of vesicles between ER and GC, blocking the exocytosis of secretory and membrane proteins. Our findings suggest that in the presence of BFA, the ER is divided into subcompartments that can be distinguished by their accessibility to assembled HA molecules.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00542-03-LVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Processing of Viral Proteins for T Cell Recognition

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. W. Yewdell	LVD, NIAID
	J. R. Bennink	LVD, NIAID
Others:	J. Cox	LVD, NIAID
	L. Eisenlohr	LVD, NIAID
	F. Esquivel	LVD, NIAID
	C. Lapham	LVD, NIAID
	G. Russ	LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.00	5.25	.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Class I molecules of the major histocompatibility complex class (MHC) consist of a highly polymorphic heavy chain complexed to B_2-microglobulin. Class I molecules are expressed on virtually all cell types. Their sole function is to bind antigens and present them to T cells bearing CD8 molecules. These T cells are known as cytotoxic T lymphocytes (CTLs) due to their ability to lyse histocompatible cells in an antigen specific manner. CTLs play a critical role in eradicating intracellular pathogens and tumors. On the negative side, they are involved in organ rejection, and in many autoimmune dyscrasias.

There has been rapid progress in understanding the physical nature of the antigen-class I complex and in how antigens become associated with class I molecules in cells. Based in part on results from this laboratory, it is now apparent that antigens present in the cytosol are tranlocated into the exocytic compartment where they bind class I molecules which carry them to the cell surface for CTL recognition.

In the past year we have continued our studies on antigen processing, which can be defined as the structural modification and trafficking of protein antigens that enable the determinants recognized by CTLs (often buried in native proteins) to interact with MHC molecules in the proper subcellular compartment. We have focused on the following questions. In which exocytic compartment does association with class I molecules occur? Are there signals for targeting proteins into the cytosolic antigen processing pathway? Are there cellular proteins which function to facilitate the association of class I molecules with antigen? Can extracellular proteins be targeted to the cytosolic antigen processing pathway?

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00563-01 LVD

PERIOD COVERED October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Production and Characterization of gp160-Specific Monoclonal Antibodies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. Yewdell	LVD, NIAID
Others:	J. Bennink	LVD, NIAID
	F. Esquivel	LVD, NIAID

COOPERATING UNITS (if any)
P. Johnson, Georgetown University, 12441 Parklawn Drive, Rockville, MD 20852

LAB/BRANCH
Laboratory of Viral Diseases

SECTION

INSTITUTE AND LOCATION
NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Terminated.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00564-02 LVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of a Parvovirus Vectors that Regulates Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. A. Rose	Section Head	LVD, NIAID
Others:	S. Chatterjee	Visiting Associate	LVD, NIAID
	K. Wong	Medical Staff Fellow	LVD, NIAID

COOPERATING UNITS (if any)

P. Johnson, Research Associate Professor, Geogetown University; V. Hirsch, Research Associate Professor, Georgetown University

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.7	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study was designed to develop adeno-associated virus (AAV)-based vectors that could down-regulate expression of specific viral genes by mediating the production of appropriate antisense RNA molecules. Among targets chosen for study are certain early viral gene products which are absolutely necessary for productive viral infection. A series of AAV-based vectors have been created in which the endogenous AAV promoters and coding sequences were replaced with a polylinker and several strong constitutive and inducible heterologous promoters. An encapsidation system has been developed in order to produce high titres of infectious recombinant virus. The induction of resistance to H1V-1 infection by such vectors is currently being studied, and present results indicate that a 95% reduction in H1V replication can be achieved. Methods used include: gene cloning, virus production, transfection of cells, radio-immunoprecipitation, immunofluorescence, RNA and DNA hybridization techniques.

LABORATORY OF INTRACELLULAR PARASITES
Rocky Mountain Laboratories
Hamilton, Montana
1990 Annual Report
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Annual Report
Laboratory of Intracellular Parasites
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1989 to September 30, 1990

Research Highlights

LICP scientists study infectious diseases caused by obligate intracellular bacterial pathogens. *Chlamydia trachomatis* and *Rickettsia rickettsii* infections are those studied primarily with research emphasis being directed towards defining and characterizing parasite surface components in relation to pathogenicity, virulence, and host immunity. The mechanisms used by intracellular parasites to infect their eucaryotic hosts and evade intracellular host defenses are being investigated. A major emphasis is placed on the identification and molecular characterization of parasite surface components that promote adhesion and invasion of host cells since they are logical targets for the development of experimental vaccines. Surface components that function as adhesions are being antigenically characterized at the molecular level in attempts to formulate rational approaches for the development of experimental vaccines to prevent or control infections caused by these intracellular pathogens; particularly sexually transmitted infections caused by *C. trachomatis*. Animal models are being used to define the role of host immunity in mediating chlamydial disease pathology. Chlamydial antigens which evoke deleterious immune responses are being characterized and the immune response(s) which promote immunopathology are being studied.

Chlamydia trachomatis: LICP scientists in the Chlamydial Diseases Section under the direction of Harlan Caldwell have made considerable progress in research investigations involving chlamydial disease pathogenesis and the identification and characterization of surface antigens which show significant promise for the development of an experimental chlamydial vaccine. Hua Su and Nancy Watkins have defined the chlamydial major outer membrane protein (MOMP) as a potential chlamydial adhesion. They identified surface exposed domains of the MOMP as potential adhesion sites through the use of monoclonal antibody neutralization studies and surface proteolysis of chlamydiae by trypsin. You-Xun Zhang and Watkins have defined the topological, structural, and antigenic properties of MOMP B-cell neutralizing determinants. Using an extensive panel of well characterized monoclonal and polyclonal neutralizing antibodies they have mapped contiguous neutralizing determinants to the four variable domains of the MOMP. Both serovar-specific and broadly cross-reactive neutralizing epitopes have been identified and their antigenic structure defined at the amino acid level. Hua Su, Rick Morrison, and Watkins have identified and characterized T-helper cell antigenic determinants of the (MOMP). Using overlapping synthetic peptides representing the entire primary sequence of the MOMP in T-cell proliferation assays eight synthetic peptides were found to contain T-cell antigenic determinants. Two of these synthetic peptides were found to function as T-helper cell antigens in that they were capable of priming mice *in vivo* to produce an anamnestic IgG response specific to B-cell neutralizing determinants of the MOMP. Mice immunized with chimeric co-linear synthetic peptides containing both T-helper and B-cell neutralizing antigenic sites produced high titered antibodies specific to the MOMP B-cell determinants of the chimeric peptide. These sera were reactive with the native MOMP

molecule and neutralized chlamydial infectivity for cultured eucaryotic cells. These findings are the first to describe T-helper cell antigenic determinants of the MOMP and should provide a rational basis for the design of antigenically defined synthetic peptide immunogens (chimeric peptides containing MOMP T-helper and neutralizing B-cell epitopes) that can be evaluated as experimental chlamydial vaccines in sub-human primates. Future studies on MOMP T-cell determinants will focus on defining the immune response to chimeric T-B cell peptides in congeneic mice differing at H-2 to ascertain if these T-cell antigens are capable of interacting with different MHC alleles. Such studies will define the MHC restriction in the immuno-responsiveness to the MOMP T-helper cell determinants which will be critical in evaluating their potential for vaccination in genetically diverse populations. Scott Manning has persued studies aimed at developing infectious bacterial and viral vectors carrying selected recombinant chlamydial MOMP gene segments. Infectious vectors have been selected on their ability to target mucosal immune responses to recombinant MOMP. Manning has expressed the complete MOMP in vaccinia virus. Mice immunized with recombinant vaccinia virus produced high titered antibodies specific to the MOMP. Future studies are planned to access the protective efficacy of recombinant vaccinia virus expressing the MOMP in animal models of chlamydial infection. Richard Morrison demonstrated that a non-infectious detergent extract of chlamydiae elicits a delayed hypersensitivity (DH) response when applied to the conjunctivae of guinea pigs that had recovered from a previous ocular chlamydial infection. These results provided the first direct evidence for immunopathologic contributions to chlamydial disease. Characterization of the antigen involved in eliciting this deleterious immune response showed it to be a 57KDa protein. The gene encoding the 57KDa protein was cloned and sequenced and was identified as a chlamydial "heat shock protein" whose analogues appear in both procaryotic and eucaryotic cells. The recombinant chlamydial 57KDa protein was purified by immunoaffinity chromatography and the purified protein is being used to conduct detailed antigenic characterization of the 57KDa protein. Ying Yuan and Morrison have initiated epitope mapping studies of the 57KDa protein. Antibody and T-cell epitopes are being mapped using subclones of the gene expressing amino terminal truncated fragments of the 57KDa protein and overlapping synthetic peptides. Antibody mapping studies have shown that the immunodominant B-cell epitopes of the protein are located at the amino and carboxyl terminal ends of the molecule. Collaborative studies are underway to examine the T-cell response to the 57KDa protein in patients with blinding trachoma, chlamydial pelvic inflammatory disease, and Reiter's syndrome to define the T-cell response to this antigen in individuals manifesting serious disease sequelae following chlamydial infection. It also planned to clone reactive T-cells from these individuals and to map the immunodominant T-cell antigenic determinants of the 57KDa protein recognized by human cloned T-cells.

Rickettsia rickettsii: Work in the rickettsial unit continues to focus on the structure and immunobiology of *R. rickettsii.* Immunodominant surface antigens of approximately 120 and 155 KDa have been identified and these antigens are being characterized at the molecular level. S. Vishwanath cloned the gene encoding the 155 KDa protein from *R. conorii* (the etiological agent of Boutonneuse Fever). Lysates prepared from recombinant *E. coli* expressing the 155 KDa protein were shown to protect guinea pigs against infectious challenge with *R. conorii* or *R. rickettsii.* Vishwanath is currently sequencing the 155 KDa gene as a preliminary step towards epitope mapping the protective regions of the protein. Bob Gilmore has cloned and sequenced the gene encoding the 120 KDa surface protein.

Work is in progress to compare the 120 KDa proteins of other members of the species at the antigenic level. Paul Policastro is investigating the expression of these proteins during rickettsial intracellular growth and is working on the development of DNA transformation systems for *rickettsiae.*

ADMINISTRATIVE REPORT

The LICP was established as a new laboratory at the RML in March of this year as a result of the division of LMSF. LMSF staff working on Chlamydial and Rickettsial projects were transferred to the LICP. One of the major changes to occur in the development of the laboratory was the recruitment of Dr. Ted Hackstadt to serve as a Unit Leader for the Rickettsial Program. Dr. Hackstadt joined LICP July 30, 1990. In the past this group of junior investigators have lacked the necessary leadership and guidance to insure a more enthusiastic and productive environment. The addition of Dr. Hackstadt to the staff will provide this leadership and it is anticipated that this administrative change will serve to greatly strengthen the rickettsial program in LICP. Other personnel changes include the departure of Drs. Zhang, Vishwanath, and Watkins who have accepted employment elsewhere. The departure of Dr. Zhang will be a major loss to the chlamydial diseases program and extensive efforts are being spent to recruit new personnel to fill his position and those vacated by Vishwanath and Watkins. Summer students include returnees Mike Jasumback and Sadie Honey along with new comers Kathy Olson, Gwendolyn Lenk, and Jennifer Rosquist. The following individuals were invited to present seminars: Dr. Gerald Byrne (University of Wisconsin), Dr. Helmut Brade (Max-Planck, Berlin), and Dr. Timothy Hirst (University of Leicester, UK), Dr. Robert Massung (University of Florida, Gainesville, Florida).

HONORS AND AWARDS

NIH Honor Awards:

H. Caldwell -	NIH Director's Award
Y. Zhang -	NIH Merit Award

Journal Editorial Boards:

H. Caldwell -	Infection and Immunity (Invited Reviewer) Journal of General Microbiology (Invited Reviewer) Journal of Bacteriology (Invited Reviewer)
R. Morrison -	Infection and Immunity (Invited Reviewer) Journal of Bacteriology (Invited Reviewer)

Professional Posts:

H. Caldwell -	Faculty affiliate, Division of Biological Sciences (Microbiology), University of Montana, Missoula, Montana.
R. Morrison -	Faculty affiliate, Division of Biological Sciences (Microbiology), University of Montana, Missoula, Montana.

Invited Lectures and Participation in Meetings and Symposia:

H. Caldwell -	Scientific Committee, Proceedings of the Seventh International Symposium on Human Chlamydial Infections, Harrison Hot Springs, British Columbia, Canada, June 24-29, 1990. Workshop on the Development of a Trachoma vaccine, Session Chairman, Easton, Maryland, October 2 - 6, 1989.
R. Gilmore -	American Society for Rickettsiology and Rickettsial Diseases, Diamond Point, N.Y., Participant.
R. Morrison -	Montana State University, Department of Microbiology Seminar Program, Bozeman, Montana (1989). The Edna McConnell Clark Foundation: Trachoma Task Force Meeting, Easton, Maryland (1989). University of Texas Health Science Center at San Antonio, Department of Microbiology, San Antonio, Texas (1990).

Co-chairman, Immunobiology of Chlamydial Infection, Seventh International Symposium on Human Chlamydial Infection, Harrison Hot Springs, British Columbia, Canada (1990).

P. Policastro - American Society for Rickettsiology and Rickettsial Diseases, Diamond Point, N.Y., Participant.

H. Su - American Society for Microbiology, Los Angeles, California.

S. Vishwanath - Department of Microbiology, Montana State University, Bozeman, Montana.

N. Watkins - Department of Chemistry, University of Toledo, Toledo, Ohio.
CBER Food and Drug Administration, Bethesda, Maryland.
Department of Life Sciences, Indiana State University, Terre Hucle, Indiana.
Department of Biochemistry, University of Minnesota Medical Shcool, Duluth, Minnesota.
Poster presentation, Proceedings of the Seventh International Symposium on Human Chlamydial Infections, Harrison Hot Springs, British Columbia, Canada, (1990).

Y. Yuan - American Society for Microbiology, Los Angeles, California.

Other Activities:

H. Caldwell - NIAID Study Section, Bacteriology and Mycology, Ad hoc member.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00216-10 LICP

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunology of Chlamydial Surface Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H.D. Caldwell	Chief	LICP, NIAID
Others:	H. Su	Visiting Associate	LICP, NIAID
	Richard Morrison	Senior Staff Fellow	LICP, NIAID
	You-Xun Zhang	Visiting Associate	LICP, NIAID
	Nancy G. Watkins	Senior Staff Fellow	LICP, NIAID
	Scott Manning	IRTA Fellow	LICP, NIAID
	Scott Stewart	Microbiologist	LICP, NIAID
	Jim Simmons	Bio. Lab. Tech.	LICP, NIAID

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Laboratory of Intracellular Parasites, Hamilton, Montana 59840

SECTION

Chlamydial Diseases

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.3	3.7	1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The major focus of this project is the development of a vaccine to prevent or control infections caused by *Chlamydia trachomatis.* The chlamydial major outer membrane protein (MOMP) is considered to be important in the development of protective immunity to chlamydial infection. Thus, current vaccine strategies are focused on the development of recombinant or synthetic peptide MOMP vaccines that can be target to induce mucosal immunity. We have undertaken an extensive effort aimed at characterizing the antigenic properties of the MOMP at the molecular level to identify immunologically relevant structures of this protein that may have utility for the rational design of a chlamydial vaccine. Previous efforts were focused on characterizing immunodominant neutralizing B-cell epitopes of the MOMP. Serovar-specific and broadly cross reactive subspecies-specific neutralizing sites were mapped to contiguous epitopes located in within the variable domains (VD) of the protein. In this year, we have undertaken studies to characterize T-helper cell antigenic determinants of the MOMP that function in inducing B-cells to produce antibody specific to neutralizing B-cell epitopes. Two regions of the MOMP were shown to contain Th-cell epitopes capable of directing B-cell clones to produce antibody specific to immunodominant neutralizing sites. Chimeric peptides containing both Th- and B-cell epitopes were synthesized and shown to be highly immunogenic. Immunization of mice with the chimeric peptides induced high titered antibodies that reacted with the native MOMP and were neutralizing *in vitro*. The Th-cell epitopes under study are apparently "promiscuous" since strains of congeneic mice differing at H-2 are capable of responding to the chimeric peptide immunogens. Evaluation of the immunogenicity and vaccine efficacy of the chimeric peptides in sub-human primates will be initiated in the upcoming year.

We are also working on the construction of infectious vectored chlamydial vaccines. Towards these goals we have expressed the complete MOMP protein in vaccinia virus and have evaluated the immunogenicity of the recombinant virus in mice. The results are promising; mice infected with recombinant vaccinia virus expressing the MOMP induced high titered antibodies specific to the protein. We plan to evaluate the protective efficacy of the recombinant virus in animal models of chlamydial infection in the upcoming year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 AI 00441-06 LICP

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Rickettsia rickettsii Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ted Hackstadt	Expert	LICP, NIAID
Others:	S. Vishwanath	Senior Staff Fellow	LICP, NIAID
	P.F. Policastro	Senior Staff Fellow	LICP, NIAID
	R.D. Gilmore	Senior Staff Fellow	LICP, NIAID
	R.E. Mann	Bio. Lab. Tech.	LICP, NIAID

COOPERATING UNITS *(if any)*
Burt E. Anderson, Centers for Disease Control, Atlanta, Georgia 30333

LAB/BRANCH
Laboratory of Intracellular Parasites, Hamilton, Montana 59840

SECTION
Ricketsial Diseases

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	3.2	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Work in the Rickettsial Diseases Section has, for the last several years, focused on the structure and immunobiology of *Rickettsia rickettsii*, the etiologic agent of Rocky Mountain spotted fever, and related species with that genus. Major surface antigens of approximately 120 and 155 kDa have been identified. Monoclonal antibodies to these major surface proteins have been produced and used to characterize the antigenic diversity of the homologous proteins from distinct geographic isolates of *R. rickettsii* and closely related species. Certain of these monoclonal antibodies recognize critical surface structures and neutralize rickettsial infectivity. The potential of the 155 kDa protein as a protective immunogen has been further demonstrated by the protection of experimental animals from Rocky Mountain spotted fever by immunization with a cloned rickettsial gene product. Isolation and expression of the homologous gene from *R. conorii*, the agent of Mediterranean spotted fever, confirmed the potential of that protein as an immunogen since it induced protective immunity to *R. conorii* and to a lesser degree, protected against challenge with *R. rickettsii*. The monoclonal antibody data indicate that the 155 kDa antigen possesses both shared (genus-specific) and unique (species or strain-specific) antigenic determinants. Molecular characterization of these epitopes and their roles in protective immunity, species variation, and potentially virulence, is continuing. A limiting factor in these studies is the small amount of rickettsial gene product produced by the recombinant bacteria. In attempts to circumvent this difficulty, rickettsial gene promoters are being identified and alternative hosts for the expression of rickettsial genes are being explored. In addition, a gene expressing the 120 kDa antigen has been cloned and its sequence determined. Surface exposure, regulation of expression, and antigenic diversity of this prominant protein are being similarly characterized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 AI 00518-03 LICP

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Antigenic Mapping of Chlamydial Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Nancy G. Watkins	Senior Staff Fellow	LICP, NIAID
Others:	H. Su	Visiting Fellow	LICP, NIAID
	H. D. Caldwell	Chief	LICP, NIAID
	S. Stewart	Microbiologist	LICP, NIAID
	J. Simmons	Bio. Lab. Tech.	LICP, NIAID

COOPERATING UNITS *(if any)*
NONE

LAB/BRANCH
Laboratory of Intracellular Parasites, Hamilton, Montana 59840

SECTION
Chlamydial Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.4	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The major outer membrane protein (MOMP) of *Chlamydia trachomatis* has been identified as a possible immunogen for vaccine development. The objectives of this project are to identify amino acid sequences of MOMP which are surface accessible to immunoglobulin and to determine if these sequences are involved in chlamydia-host cell attachment. Peptides containing sequences of the four variable domains (VD I, II, III, and IV) have been synthesized. These peptides have been used to define surface accessible epitopes. The B serogroup have accessible epitopes on VD II and VD IV; in contrast, the intermediate and the C serogroup have accessible epitopes on VD I and VD IV. Trypsin cleavage of VD II and VD IV of the MOMP of serotype B on the surface of viable EBs results in loss of binding of chlamydiae to HeLa 229 cells. Monoclonal antibodies to VD II and VD IV of serotype B MOMP neutralize infectivity of chlamydiae for HAK cells by preventing attachment. These data indicate that MOMP has a role in attachment of chlamydiae to host cells. MOMP appears to mediate attachment through electrostatic interactions of the hydrophilic surface accessible variable domains and through binding to a hydrophobic pocket in a conserved region of VD IV. The binding to the hydrophobic pocket is dependent upon the conformation of MOMP. Current research is focused on using overlapping synthetic peptides (8 amino acids) attached to pins to identify immunodominant regions of the VDs. Studies using sera from guinea pigs infected with *C. psittaci* strain GPIC indicate that VD I and VD IV are immunodominant during infection. From these studies, we hope to determine if surface accessible epitopes are also immunodominant during infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00519-03 LICP

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunopathogenesis of Chlamydial Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.P. Morrison	Senior Staff Fellow	LICP, NIAID
Others:	H.D. Caldwell	Chief	LICP, NIAID
	Y. Yuan	Visiting Fellow	LICP, NIAID
	S. Stewart	Microbiologist	LICP, NIAID
	J. Simmons	Bio. Lab. Tech.	LICP, NIAID

COOPERATING UNITS *(If any)*
Div. Rheumatology, Univ. Toronto, Ontario, Canada (R. Inman); London Sch. of Trop. Med. and Hyg., London, United Kingdom (D. Mabey); Dept. Med. Microbiol., Univ. Wisconsin Med. Sch., Madison, WI (G. Byrne).

LAB/BRANCH
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SECTION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.9	0.2

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Chlamydia trachomatis, an obligate intracellular pathogen of humans, primarily infects the ocular and genital mucosa to cause urethritis, cervicitis, and conjunctivitis. These infections generally resolve without adverse sequelae, but in many instances infection progresses to severe inflammation leading to blindness, infertility and arthritis. These severe sequelae are major public health concerns throughout the world. An estimated 500 million people in developing countries have trachoma and an estimated 7 million are permanantly blind as a result. Currently, there are about 4 million new cases of sexually transmitted chlamydial infections per year in the United States. Ascending chlamydial infections in women often lead to pelvic inflammatory disease, tubal blockage, infertility and ectopic pregnancy. An estimated 20,000 women per year in the US are thought to become involuntarily infertile as a result of chlamydial-induced pelvic inflammatory disease. Because chlamydial infections are a major public health concern a better understanding of the pathogenesis of the diseases caused by *C. trachomatis* is needed. The pathogenesis of chlamydial disease is thought to be immunologically mediated. Using animal models of chlamydial infection we have demonstrated that a delayed hypersensitivity response to a chlamydial 57kD protein may be involved in the pathogenesis of chlamydial disease. This chlamydial protein belongs to the family of widely conserved 60kD stress-response proteins found in eucaryotes and procaryotes. Because of the proposed importance of the immune response to this protein and the development of the serious sequelae associated with chlamydial disease we are characterizing its antigenic properties. Sequence analysis of the genes encoding the 57kD protein from *C. trachomatis* and the animal pathogen *C. psittaci* reveals that this protein is 94% identical among chlamydial species and > 50% identical to homologous proteins from other procaryotes and eucaryotes. Preliminary data on the mapping of immunoreactive determinants of this protein reveal that the amino terminus and carboxyl terminus are immunoreactive. More complete mapping of immunoreactive antibody determinants is underway. We have been successful in purifying homogeneous preparations of the 57kD protein and can now begin studies to evaluate the T-cell responses to this protein, and the mapping of T-cell reactive epitopes. These studies will provide information useful to understanding the pathogenesis of chlamydial disease.

LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
Rocky Mountain Laboratories
Hamilton, Montana
1990 Annual Report
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Annual Report
LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1989, to September 30, 1990

RESEARCH HIGHLIGHTS

LMSF scientists mainly focus on disease-causing microbes and their interactions with eukaryotic hosts. General goals are to decipher the infectious mechanisms employed by the microorganisms and those host responses that might be exploited for preventing or treating infections. Special attention is paid to genetic changes that produce variations in microbial constituents, especially those that lead to evasion and/or avoidance of recognition and destruction by the infected host. During FY 1990, LMSF scientists and technical staff have studied one or several of these facets of *Neisseria gonorrhoeae*, *Borrelia burgdorferi*, group B streptococci, and human immunodeficiency virus (HIV).

Neisseria gonorrhoeae **(gonococci) and gonorrhea:** Previous studies by LMSF staff revealed remarkable variability of gonococcal pili in the contexts of experimental infections of male volunteers. The genetic mechanisms responsible for this variation are being explored by Stuart Hill. His results indicate that such variation is encoded in the sequences of the pilin genes in gonococci. A multitude of direct and indirect nucleotide repeats reside in both the complete, expressed pilin gene (*pil*E) and the 16 - 20 silent, partial genes (*pil*S) of the gonococcal chromosome. These repeats direct the formation and extent of *pil*E deletions. Many of the deletions are "repaired," apparently on a *pil*S template, and produce to pilus "antigenic variation." Others are not repaired immediately and give rise to pilus "phase variation." The above-noted experimental human gonorrheal infections were initiated with gonococci that do not express outer membrane Opa proteins (for their colony opacity-association, previously called protein II); but the vast majority of organisms reisolated from the infected volunteers express one, another, or several Opa constituents. The entire repertoire of Opa-encoding *opa* genes were cloned and sequenced by Kolari Bhat. The Opa proteins expressed by each *opa* gene have been assigned. The molecular events and sequence parameters that effect the on/off switching of *opa* elements are being explored by a combination of approaches. Each *opa* contains a leader peptide-encoding portion that is largely comprised of pentameric repeats (CTTCT) whose number changes coincident to on/off switches in expression. Bob Belland has found that this repeat region arranges into an "H-form" or triple stranded DNA configuration, and this appears to dampen the tendency of an *opa* to change the number of its repeats and switch expression status. Belland is also examining the role of DNA gyrase or topoisomerase II in *opa* switching; in collaboration with Wai Mun Huang (University of Utah), he identified, cloned, and sequenced the gonococcal gyrase genes which are being mutated *in vitro* for reintroduction into gonococci. Gonococci are highly unusual among bacterial species examined to date in carrying two *gyr*A genes (plus a single *gyr*B element). John Swanson has found that the length of the *opa* repeat region directly correlates with the tendency of this gene to undergo on/off switching. He has also studied lipooligosaccharide (LOS) mutants of gonococci as it influences the surface exposures and biological reactivities of Opa proteins. His studies indicate that only some LOS variant molecules are suitable for addition of sialic acid residues which greatly influence the surface properties of gonococci. He is defining the affects of LOS variation and sialylation, pilus formation, and Opa expression on gonococcal surface charge or ζ-potential. Steven Fischer has begun to explore these changes as they relate to interactions between gonococci and human neutrophils.

***Borrelia burgdorferi* and Lyme disease:** Discovery of *B. burgdorferi* as the causative agent of Lyme disease has been followed by intense investigation of this bacterium. But major problems persist in its diagnosis due to difficulties in isolation of the causative agent, growth *in vitro*, etc. And major gaps exist in many aspects of our understanding of Lyme disease, including mechanisms of pathogenesis, variation of *B. burgdorferi* constituents that interplay with defense elements of the host, etc. Patti Rosa has devised a sensitive, specific assay based on polymerase chain reaction that can detect small numbers of *B. burgdorferi*. This assay allowed definition of two major groups of these organisms and has permitted detection of organisms in tissue biopsies. Rosa is also exploring mechanisms responsible for rearrangements in genes that encode two major outer membrane proteins (OspA and OspB) that are dominant immunogens of these borreliae. Kit Tilly is examining the pathogenic role(s) of *B. burgdorferi* heat-shock genes that may relate to survival and growth of these spirochetes in ticks (ambient temperature) and mammalian hosts (37°C). Tilly is also constructing shuttle vectors and exploring transfer of genes into *B. burgdorferi*, important preludes to her genetics studies of this organism.

Immunoglobulin biology: In spite of the remarkable accumulation of information on immunoglobulin structure and function, there are inadequate therapeutic applications against many infectious diseases. Seth Pincus and co-workers are addressing several medically-important areas that may be amenable to therapeusis with immunoglobulin-based reagents. Pincus has constructed a series of immunotoxins based on HIV-directed (anti-envelope) monoclonal and polyclonal antibodies conjugated with ricin A chain. Some of these immunotoxins are highly effective in destroying HIV and HIV-infected cells *in vitro*, except as resistant HIV variants arise. Pincus then defined the envelope mutations in these immunotoxin-resistant variants and is currently exploring the utility of ricin immunotoxins that incorporate polyclonal antibodies obtained from immune sera (human and laboratory animals). Carol Horgan has engineered the construction of immunoglobulin molecules with human mu, gamma-1, or gamma-4 constant region genes ligated to murine genes of variable regions of antibodies directed against TGAL (a synthetic oligopeptide). These chimeric antibodies have proved highly useful in study of the effector functions of antibodies. Analogous antibody gene constructs directed against group B streptococci are being made by Horgan, Kamanga-Sollo, and Pincus for potential therapeutic use against group B streptococcal sepsis in human neonates. Pincus is also exploring the idiotypic network with immunotoxins constructed from anti-idiotype antibodies conjugated to ricin A chain.

Interspecies genetic transfers: Genetic exchanges between highly-related microorganisms are directly relevant to such topics as acquisition of antibiotic resistance by bacteria that are pathogenic for humans and other animals. Clear examples of DNA transfer are also known in plant pathology as well in eukaryotic and prokaryotic virus systems. But, in general, little is known about the potential for dissimilar life forms to exchange genes. Jack Heinemann demonstrated previously that bacterial (*E. coli*) genes can be transferred into yeast (*Saccharomyces cerevisiae*) via conjugation, and his current work represents both direct expansions of those studies as well as explorations of the mechanisms whereby such genetic transfers are permitted. He is constructing plasmids with which he can evaluate the function of virulence-related genes from *Agrobacterium tumefaciens* in conjugation between *E. coli* and yeast. These constructs should allow a detailed study of the conjugation process. In addition, Heinemann is exploring gene transfer into pathogenic organisms (gonococci in collaboration with Belland, and Lyme disease spirochetes with Tilly). Along with their implications for human disease, his studies will undoubtedly shed new light on traditional concepts of speciation and evolution.

ADMINISTRATIVE REPORT

Personnel changes during FY'90 were extensive since the creation of the Laboratory of Intracellular Parasitism (LICP) incorporated several former LMSF staff members, as follows: H. Caldwell (Chief); Senior Staff Fellows R. Morrison, P. Policastro, S. Vishwanath, and N. Watkins; IRTA Fellows S. Manning and R. Gilmore; Visiting Fellow Y. Yuan; Visiting Associates Y-X Zhang and Su Hua; technicians S. Stewart, J. Simmons, and R. Mann; guest workers J. Sager, Ya-Qi Zhu, K. Lyng. Newly-arriving to LMSF were Kathryn Tilly (Senior Staff Fellow), Ernest Kamanga-Sollo and Chen Tie (Visiting Fellows), Jack Heinemann (IRTA Fellow), and Steven Fischer (Research Associate). Summer students in LMSF included the following: Kathy Wells (Creighton University School of Medicine), Conley Lynch (Carroll College), Donald Hoffman (University of Montana), Jerry Ostheimer (Dartmouth University), and Bethany Tucker (Middlebury College). The LMSF seminar schedule included the following individuals: U. Munderloh and T. Kurtti (University of Minnesota), M. Blake (Rockefeller University), M. Gordon (LIG, NIAID), R. May (University of Texas Southwest), W-M. Huang and S. Casjens (University of Utah), J.M. Koomey (University of Michigan), and A. Edmundson (Harrington Cancer Center). Recruiting visits to RML were made by the following: A. Camilli (University of Pennsylvania), S. Ecklund (Bowman-Gray Medical School), S. Fischer (University of Pennsylvania), D. G. Ennis (DuPont Experimental Station), R. Ankenbauer (University of Washington), E. Anderson (University of Oregon), G. Laible (Max-Planck, Berlin).

HONORS AND AWARDS

Journal Editorial Boards:

J. Swanson - Infection and Immunity
S. Pincus - medical advisory board for Arthritis Today

Manuscripts were reviewed by LMSF staff for the following journals: Canadian Journal of Microbiology, Cell, Infection and Immunity, Journal of Bacteriology, Journal of Biological Chemistry, Journal of Clinical Investigation, Journal of Clinical Microbiology, Journal of General Microbiology, Journal of Immunology, Journal of Infectious Diseases, Journal of Rheumatology, Microbial Pathogenesis, Proceedings of the National Academy of Sciences USA, Science, and Sexually Transmitted Diseases.

Professional Posts:

S. Pincus - Adjunct Associate Professor of Internal Medicine, University of Utah, Salt Lake City, Utah

Invited Lectures and Participation in Meetings and Symposia:

R. Belland - Seventh International Pathogenic Neisseria Conference, West Berlin, Germany

R. Cole - Montana Biotechnology Symposium, Bozeman, Montana

J. Heinemann - University of British Columbia, Vancouver, British Columbia, Canada
Montana State University, Bozeman, Montana
Columbia University, New York, New York
Keynote address, International Conference on Gene Transfer Mediated by Bacterial Plasmids, Banff, Alberta, Canada

S. Hill - Seventh International Pathogenic Neisseria Conference, West Berlin, Germany

C. Horgan - Montana Biotechnology Symposium, Bozeman, Montana
Arthritis Foundation Fellows Conference, Amelia Island, Florida
Miami Biotechnology Winter Symposia, Miami, Florida

S. Pincus - Targeted Cellular Cytotoxity Meeting, Annapolis, Maryland
UCLA Symposia on Molecular and Cellular Biology; HIV and AIDS: Pathogenesis, Therapy and Vaccine, Keystone, Colorado
Harvard Medical School, Cambridge, Massachusetts
Repligen Corporation, Cambridge, Massachusetts
Laboratory of Tumor Cell Biology, NCI, NIAID, Bethesda, Maryland

P. Rosa - Abbott Laboratories, Chicago, Illinois
Fourth International Conference on Lyme Borreliosis, Stockholm, Sweden
Southwest Michigan Lyme Conference, Battle Creek, Michigan
National Multiple Sclerosis Society, Montana State Convention, Missoula, Montana
The International Northwestern Conference on Diseases in Nature Communicable to Man, Hamilton, Montana

J. Swanson - NIAID Introduction to Biomedical Research Program, Phase I, Bethesda, Maryland
Visiting Scholar, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina
Seventh International Pathogenic Neisseria Conference, West Berlin, Germany
Consultant, MID, NIAID, Bethesda, Maryland

Other Activities:

S. Pincus - Co-chairman of research grants review for Arthritis Foundation Fellowship.

J. Swanson - Reviewed research grants for National Science Foundation, Washington, DC

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 AI 00193-11 LMSF

PERIOD COVERED
October 1, 1989, to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gonococcal Surface Components: Structure and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Swanson	Chief	LMSF, NIAID
Others:	K. Bhat	Senior Staff Fellow	LMSF, NIAID
	R. Belland	Visiting Associate	LMSF, NIAID
	S. Hill	IRTA Fellow	LMSF, NIAID
	S. Fischer	Research Associate	LMSF, NIAID
	O. Barrera	Microbiologist	LMSF, NIAID
	S. Morrison	Microbiologist	LMSF, NIAID

COOPERATING UNITS *(if any)*
Wai-Mun Huang, University of Utah, Salt Lake City, Utah

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.25	4.25	3.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The pathogenic personality of gonococci (Gc) is largely determined by their surface constituents, including pili, Opa proteins, and lipooligosaccharide (LOS). Pili are critical for virulence, presumably because they adhere gonococci to the mucosal sites of infection; but these critical surface organelles change at high frequencies due to sequence changes in their expressed structural gene (*pil*E). Expression of Opa or opacity-associated proteins of the outer membrane is a consistent feature of gonococci reisolated from male urethritis. A single strain has a repertoire of several different *opa* genes. The function of the Opa family of proteins is unclear except for their binding gonococci together and influencing both attachment and detachment of organisms from tissue culture cells *in vitro*. LOS structure influences the susceptibility of gonococci to killing by normal human sera. We are trying to define the genetic mechanisms responsible for pilus structure variation and for "on/off" switching of *opa* genes. The degree of structural heterogeneity among the Opa repertoire of one strain has been evaluated by molecular cloning and sequencing all its *opa* elements. A topoisomerase (gyrase) of gonococci has been cloned, is being sequenced, and will be evaluated regarding its role in these pilin and Opa changes. LOS variation and sialylation (substitution of neuraminic acid residues) is being studied in terms of their affects on gonococcal surface properties and on the interactions of gonococci with host factors and cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 AI 00516-03 LMSF

PERIOD COVERED
October 1, 1989, to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunoglobulin Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

	Name	Title	Affiliation
PI:	S. H. Pincus	Expert	LMSF, NIAID
Others:	C. J. Horgan	Senior Staff Fellow	LMSF, NIAID
	E. I. P. Kamanga-Sollo	Visiting Fellow	LMSF, NIAID
	R. L. Cole	Chemist	LMSF, NIAID

COOPERATING UNITS *(If any)*

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Laboratory of Microbial Structure and Function, Hamilton, MT 59840

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INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	2.7	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The goal of this work is to understand the structure and function of the immunoglobulin molecule. The work is being performed so that this understanding will be applied to the development of antibodies for human therapy.

A. Therapeutic systems. 1. Antibodies to surface antigens of group B streptococci have been demonstrated to have protective efficacy in a model of neonatal sepsis. We have studied the efficacy of the interleukin GM-CSF both *in vivo* and *in vitro* when applied in combination with the antibody. 2. The *in vitro* efficacy of anti-HIV envelope antibodies coupled to ricin A chain has been studied. Monoclonal and polyclonal antibodies directed against different epitopes have been tested. Biological variants of HIV that escape immunotoxin action have been characterized and the mechanism of immunotoxin escape has been studied.

B. Genetically engineered antibodies. Vectors have been prepared carrying human mu, gamma-1 and gamma-4 genes. Mutant constructs have been created with altered constant regions. These constructs have been ligated to variable region genes from antibodies to the synthetic peptide (Tyr,Glu)-Ala--Lys. Antibodies were expressed and the function of the engineered antibodies has been studied in immune complexes. We are currently cloning variable region genes from anti-group B streptococcal antibodies which will be ligated into the constant region vectors and expressed.

C. Idiotypy. The idiotypic network induced in mice by immunization with (Tyr,Glu)-Ala--Lys has been explored. The immune response of transgenic mice that overexpress the heavy chain of antibody 1 has been explored. An anti-idiotypic antibody has been coupled to ricin A chain. The *in vivo* effect of this anti-idiotypic immunotoxin is being explored.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 AI 00549-02 LMSF

PERIOD COVERED
October 1, 1989, to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathogenesis of Infection with the Lyme Disease Spirochete, *Borrelia burgdorferi*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. A. Rosa	Senior Staff Fellow	LMSF, NIAID
Others:	K. L. Tilly	Senior Staff Fellow	LMSF, NIAID
	D. M. Hogan	Microbiologist	LMSF, NIAID

COOPERATING UNITS *(if any)*
T. G. Schwan, NIAID, RML, Laboratory of Vectors and Pathogens
W. J. Simpson, NIAID, RML, Laboratory of Vectors and Pathogens

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	1.7	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are developing the essential components of a system with which to investigate the pathogenesis of Lyme disease and the basic biology of the causative agent, *Borrelia burgdorferi*. Sensitive and accurate detection of organisms is necessary in order to compare their presence (or absence) with disease symptoms and to facilitate an accurate diagnosis of infection. Characterization of key structural and regulatory elements of an organism is requisite to identifying potential pathogenic mechanisms. Ultimately, critical proteins can be genetically manipulated and reintroduced and the outcome on infection and disease assessed.

1. Detection: A sensitive and specific PCR assay has been developed from a chromosomally encoded *B. burgdorferi* DNA sequence. Sequence comparisons permit the design of broadly reactive but *burgdorferi*-specific primers. A modified protocol using "nested" primers has increased the sensitivity and specificity in the presence of excess eukaryotic DNA. When tested against 31 different *B. burgdorferi* isolates of broad geographic and biological origins, all were detected.

2. Molecular Characterization: Several features of *B. burgdorferi* are being addressed. Classification of isolates by reactivity with different sets of PCR primers subdivides *burgdorferi* strains into 2 groups. Analysis and sequencing of the genes encoding 2 major outer surface proteins has identified potential mechanisms for variation in expression of these immunodominant components. Modification of a plating protocol has provided a more rapid and efficient means of cloning organisms and has permitted the clonal analysis of variants. Isolating and studying genes whose expression is increased after a temperature upshift (heat shock genes) will provide information about their roles in cell growth, pathogenesis, plasmid replication and adaptation to stress.

3. Gene Transfer: In order to allow genetic analysis of *B. burgdorferi*, two methods for gene transfer into *Borrelia* are being developed. Vectors have been constructed to allow transferred DNA to integrate or transpose into the chromosome. Parameters for transformation using electroporation are being tested. Conditions for the alternative to electroporation, mating with *E. coli*, are being established.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 AI 00568-01 LMSF

PERIOD COVERED
October 1, 1989, to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Basis and Extent of Microbial Sex

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. A. Heinemann	IRTA Fellow	LMSF, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

I am interested in the molecular mechanisms by which bacteria transfer DNA to other organisms. I am concentrating on bacterial conjugation both between pairs of prokaryotes and between prokaryotes and eukaryotes and on systems that practice natural transformation. The knowledge gained about these processes could eventually be used to determine the role of DNA transmission in the pathogenesis of microbial infections and in the spread of antibiotic resistance determinants among pathogens.

Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
1990 Annual Report
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Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1989 to September 30, 1990

ADMINISTRATIVE REPORT

The following staff changes occurred at LPVD this past year:

Arrivals:

Dr. Wendy Maury, Staff Fellow, from National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Molecular Microbiology, Bethesda, MD, working with Dr. Bruce Chesebro.

Dr. Hiroyuki Kanno, Visiting Fellow, from Department of Pathology, Tohoku University School of Medicine, Sendai, Japan, working with Dr. Marshall Bloom.

Dr. Brian Danneman, Medical Officer, from Department of Medicine, Stanford University School of Medicine, Palo Alto, CA, working with Dr. Bruce Chesebro.

Dr. Louis F. Qualtiere, Special Volunteer, from Department of Microbiology, University of Saskatchewan, Saskatoon, Saskatchewan, working with Dr. John Portis.

Departures:

Dr. Masaaki Miyazawa, Visiting Associate, returning to work in the Department of Pathology, Tohoku University School of Medicine, Sendai, Japan.

Dr. Shiro Mori, Visiting Fellow, returning to work in the Department of Pathology, Tohoku University School of Medicine, Sendai, Japan.

Dr. Kathryn Neary, IRTA, going to Fred Hutchinson Cancer Research Center, 1124 Columbia, Seattle, WA 98104

Summer students were David Stokesberry from Oklahoma State University, Stillwater, OK; Bradley Berry from Caroll College, Helena, MT; and Michelle Loftis from Eastern Washington College, Cheney, WA; and Troy Sullivan from University of Medical College of Georgia, Augusta, GA.

Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1989 to September 30, 1990

LPVD - Significant Accomplishments - 1990

1. HIV genomes pseudotyped by packaging in the envelope of mouse retroviruses were able to infect CD4-negative human and mouse cells.

2. Some CD4-positive human cell lines were resistant to entry and infection by HIV. This data indicates that in addition to CD4 other cellular molecules are required for successful HIV infection.

3. Friend mouse retrovirus sequences in the U5-gag-pol region were found to be required for successful virus induction of early hemolytic anemia. Friend and Moloney virus sequences in the envelope and U3 regions usually thought to influence tissue tropism and expression did not differ in their effect on early hemolytic anemia.

4. Recombinant vaccinia viruses expressing the amino-terminal half of the gag (core) polyprotein induced protective immunity to Friend murine retrovirus.

5. Scrapie agent propagated *in vitro* in mouse neuroblastoma cells was sensitive to inactivation by prolonged protease digestion, but was resistant to a variety of nucleases.

6. The proteinase-K-resistant form of prion protein is associated with the presence of the scrapie agent both *in vivo* and *in vitro*. This abnormal protein was shown not to be located on the cell surface of scrapie-infected neuroblastoma cells.

7. Rapid induction of neurodegenerative disease by a chimeric mouse retrovirus was found to be strongly influenced by gene sequences between the U5 and gag regions.

8. Age-related resistance to CNS disease induced by a mouse retrovirus appears to be related to developmental loss of susceptibility to infection rather than development of a protective immune system.

9. Mutation rate of mouse retrovirus has been carefully analyzed and was found to be 20-fold lower than previous published estimates.

10. Protective immunity to rabies virus infection was induced by recombinant vaccinia viruses expressing either glycoprotein or nucleoprotein antigens.

11. Induction of paralysis in mice by rabies virus was found to be dependent on the presence of normal Thymus-derived lymphocytes.

12. *In vivo* depletion of helper T lymphocytes abolished ability of both SJL and BALB/c mice to recover spontaneously from rabies virus infection.

13. *In situ* hybridization demonstrated mRNA and replicating forms of Aleutian disease virus DNA in macrophages and dendritic cells of mink lymphoid organs.

14. Construction of chimeric recombinant Aleutian disease viruses identified the central portion of the viral genome as one region influencing ability to replicate *in vitro*.

15. Recombinant vaccinia viruses expressing Aleutian disease virus proteins, VP1 and VP2, induced production of parvoviral particles *in vitro*.

16. Steroid hormone control of female protein expression was different in Armenian and Syrian hamsters. In contrast to previous results in Syrian hamsters, in Armenian hamsters female protein was not sex-limited and administration of estrogen induced a ***decrease*** in serum protein levels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00072-19 LPVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Host and Viral Factors in Resistance to Rabies Virus Infection in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. L. Lodmell	Scientist Director	LPVD, NIAID
Other:	Masami Sugamata	Visiting Scientist	LPVD, NIAID
	Linda L. Perry	Expert	LPVD, NIAID
	Joseph J. Esposito		CDC
	William J. Bellini		CDC

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

This is a non-clinical IIDEA project.

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The principal objectives of this study are two-fold: 1) to determine host and viral factors that influence the genetically controlled resistance of inbred mouse strains to street rabies virus (SRV) and 2) to determine the mechanism(s) by which recombinant vaccines expressing different structural proteins of rabies virus protect highly susceptible strains of mice against lethal infection.

Cytotoxic T lymphocyte (CTL) studies have shown that susceptible strains of mice inoculated with either lethal or non-lethal concentrations of SRV have identical splenic CTL activities during the course of their infections. Passive transfer experiments and CTL assays with the same population of rabies virus immune spleen cells revealed that there was no correlation between the ability of the cells to protect naive animals from infection and the capacity of the cells to express CTL activity. A direct correlation with survival did exist in the concentration of anti-rabies virus neutralizing antibody that was produced by the transferred cells and was present in the serum of the recipient animals following cell transfer and just prior to their infection with SRV.

Studies with recombinant vaccines expressing the rabies virus nucleoprotein (NP) revealed that mice vaccinated via tailscratch were well protected against a lethal rabies virus infection for up to 30 wks postvaccination. Immunological studies determined that the NP does not elicit neutralizing antibody or CTLs. Furthermore, target cells infected with recombinants expressing the NP are not lysed by rabies immune cells. To date the mechanism(s) of resistance elicited by the rabies virus NP is unknown.

Recent studies have shown that immunodeficient athymic HSDnu/nu mice did not develop rear leg paralysis following infection with SRV. In contrast, immunocompetent HSDnu/+ mice become paralyzed. HSDnu/nu mice reconstituted with $1X10^8$ normal HSDnu/+ spleen cells and challenged one day later with SRV developed rear leg paralysis. HSDnu/nu mice reconstituted with normal HSDnu/+ spleen cells treated with anti-Thy 1.2^+ plus complement prior to transfer failed to develop paralysis following SRV infection.

Histological studies revealed perivascular mononuclear cell infiltration (PMCI) in the white matter of thalamus, hypothalamus, midbrain and pons of HSDnu/nu mice that had been reconstituted with normal spleen cells and challenged with SRV. Similar PMCI was not observed in animals that received SRV only or cells only. Immunohistochemical studies revealed both $CD4^+$ and $CD8^+$ T lymphocytes and Mac-1^+ cells in areas of the brain in which PMCI was detected. Similar cells were rarely observed in the control groups. In HSDnu/nu mice that had received normal cells and SRV, a diffuse $CD8^+$ T lymphocyte infiltration was detected in the parenchymatous tissue in the identical areas in which PMCI occurred. $CD4^+$ T lymphocyte infiltration into parenchymatous tissue was never observed. We are continuing to investigate the importance of CTLs in resolving rabies infections within the CNS, the mechanisms of immunity induced by recombinant vaccines expressing the rabies NP, and the influence of hormones on the activation of virus in persistently infected animals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00074-18 LPVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Genetically Controlled Mechanisms of Recovery from Friend Virus-Induced Leukemia

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	B. Chesebro	Chief	LPVD, NIAID
Other:	M. Miyazawa	Visiting Associate	LPVD, NIAID
	M. Robertson	Medical Staff Fellow	LPVD, NIAID

COOPERATING UNITS (*if any*)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.9	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

This is a non-clinical AIDS-related project.

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The goal of this project is to understand mechanisms of host defense and immunity against retroviruses. Previous work has defined several mouse genes which influence ability of mice to recover spontaneously from Friend virus complex (FV)-induced leukemia. Some of these genes also influence ability to induce protective immunity to this disease by vaccination with viral protein in adjuvants or expressed in recombinant vaccinia viruses.

The most recent work has shown that protective immunity can be induced by both retroviral envelope and gag proteins expressed in vaccinia. Deletion of the amino or carboxy-termini of the gag polyprotein indicated that the amino-terminal half was responsible for the immune protection. This protection was weaker than that induced by the viral envelope protein, but it was successful in mice who were not capable of making a successful immune response to the envelope protein. These results suggested that both envelope and gag proteins should be considered as possible immunogens in constructing vaccines against other retroviruses. The mechanism of protection by immunization with gag protein is under further investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00085-13 LPVD

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Pathogenesis of Aleutian Disease Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. E. Bloom	Medical Officer	LPVD, NIAID
Other:	S. Mori	Visiting Fellow	LPVD, NIAID
	H. Kanno	Visiting Fellow	LPVD, NIAID

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.8	0.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

This is a non-clinical IIDEA project.

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The scope of this project is to elucidate pathogenic mechanisms involved in infections of mink with Aleutian mink disease parvovirus (ADV).

In the past year we performed detailed studies on ADV infected adult mink using immunohistochemistry and strand specific in situ hybridization. Immunohistochemistry was performed using antiserum specific for either virion or nonstructural proteins of ADV. Modifications to the in situ hybridization procedure increased the sensitivity between 10 and 100-fold, but due to the restricted nature of ADV replication in adult mink, combination immunohistochemistry and in situ hybridization was not possible.

Immunohistochemical staining of mesenteric lymph node from adult mink 10 d after infection with ADV-Utah 1 localized ADV virion antigen in both the nuclei and cytoplasm of 2 types of cells. One had the morphology of macrophages located primarily along medullary cords and the other type resembled antigen presenting follicular dendritic cells (FDC) found within germinal centers. In situ hybridization revealed evidence of replication (mRNA and replicative form DNA) in cells having an identical distribution, however, it appeared that other macrophages and FDC contained virion DNA, but not replicative intermediates (mRNA and replicative form DNA). By 60 d after infection, however, active replication was noted only in macrophages. These results suggested that cells involved with phagocytosis and antigen presentation were targets for ADV replication throughout the course of infection.

In addition, it was observed that 60 d after infection ADV replication was also occurring in renal tubular cells and glomeruli and that the replication correlated with glomerular pathology and desquamation of renal tubular cells. These studies suggested that mechanisms other than simple immune complex deposition may be involved in the genesis of the ADV renal disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00086-13 LPVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Pathogenesis of Diseases Induced by Non-Oncogenic Retroviruses

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. L. Portis	Medical Officer	LPVD, NIAID
Other:	Markus Czub	Visiting Fellow	LPVD, NIAID
	William Lynch	IRTA	LPVD, NIAID
	Stephanie Czub	Visiting Fellow	LPVD, NIAID
	Louis Qualtiere	Special Volunteer	LPVD, NIAID

COOPERATING UNITS (*if any*)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.05	3.75	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

This is a non-clinical AIDS-related project.

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The retroviruses being studied induce a non-inflammatory neurodegenerative disease in mice which is similar in pathologic features to that caused by the slow unconventional agents such as scrapie. The original viruses which induce this disease were isolated from wild mice in California and causes a paralytic disease associated with tremor after an incubation period of from 3-12 months. A series of chimeric retroviral genomes have been constructed to explore the viral sequences which are necessary for the expression of neurovirulence. In addition to the viral envelope gene which appears to target the viruses to the relevant cells in the central nervous system (CNS) and is a major determinant of the sites of pathology, sequences within the non-translated region of the viral genome have been found to be important in allowing the viruses to replicate to high levels in the CNS. The tempo of the neurodegenerative disease appears to be a function primarily of the nature of a 500 base sequence of the viral genome immediately 5' of the start codon of the structural gene for the *gag* polyprotein. This observation indicates for the first time that sequences outside the U3 region of the LTR (which contains sequences which regulate transcription) can dramatically influence a retroviral disease. The precise sequence responsible for this effect is currently under investigation. We have found that the CNS is susceptible to infection from day 1 through day 8 of post-natal life. However, after 8-10 days of age, although the mouse can be productively infected, the CNS becomes highly resistant to infection. This resistance appears to be due to the developmental program of the CNS and does not involve the immune system. We now suspect that this "window" of susceptibility is an important determinant of the incubation period of the neurologic disease. In the last year we have made a major effort to identify the sites of virus expression in the CNS at both the protein and nucleic acid level. These studies indicate that the spongiform neurodegeneration seen in the motor areas of the CNS is probably due only indirectly to virus replication. Virus replication in one part of the CNS appears to be inducing degeneration in other regions. How this occurs is a matter of intense interest in our laboratory and may bear on the mechanisms of certain neurodegenerative diseases of man.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00260-09 LPVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Endogenous and Recombinant Retroviruses in Leukemia and Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B. Chesebro	Chief	LPVD, NIAID
Other:	J. Portis	Medical Officer	LPVD, NIAID
	L. Evans	Chemist	LPVD, NIAID

COOPERATING UNITS (if any)

Dr. M. Sitbon, Hopital Cochin, Paris, France

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

This is a non-clinical AIDS-related project.

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is aimed at investigation of mechanisms of pathogenesis of murine leukemia viruses. The Friend murine leukemia helper virus (F-MuLV) induces early hemolytic anemia (EHA) three weeks post inoculation of newborn mice. In contrast, Moloney murine leukemia virus (M-MuLV) does not induce severe EHA. In order to define which portions of the F-MuLV and M-MuLV genomes were responsible for the differences in induction of EHA, recombinant retroviruses were constructed exchanging different portions of the genomes of F-MuLV and M-MuLV. The results indicated that only retroviruses containing the U5-gag-pol region of F-MuLV could induce EHA. The precise sequence in this region responsible for the pathogenesis of EHA is now being defined by construction of additional recombinant retroviruses and site-specific mutagenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00262-09 LPVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Pentraxins in Acute and Chronic Pathology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. E. Coe	Medical Officer	LPVD, NIAID
Other:			

COOPERATING UNITS (if any)

Drs. B. Canquilhem & P. Pevet, Strasbourg, France; Dr. B. Dowton, Wash. Univ. Med. School, St. Louis, MO; Dr. K. Ishak, AFIP, Washington, D.C.; Dr. U. Nilsson, Uppsala, Sweden; Dr. Mortensen, OSU, Columbus, OH; Dr. D. Johnson, U. Kansas Med. Center, Kansas City; Dr. Archie Vomachka, Marquette U., Milwaukee

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

This is a non-clinical IIDEA project.

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Syrian hamster has a peculiar sex limited serum protein, expressed as a major protein in females (therefore called Female Protein) and testosterone suppressed in males. Female Protein (FP) is a homolog of two human pentraxins, C-reactive protein (CRP) and serum amyloid P component (SAP) as shown by similar structure (pentameric) and amino acid sequence. Furthermore, FP shares function-properties with both human pentraxins such as Ca^{++} dependent phosphorylcholine binding, complement fixation, acute phase responsiveness (characteristics of CRP) and also is a constituent of amyloid (characteristic of AP). Indeed, high serum levels of FP occurring naturally (as in female) or experimentally (as in hormonally treated male) are directly associated with deposition of amyloid. We have obtained further information which indicates that high serum levels of FP are a primary cause of hamster amyloidosis. Thus, females treated with testosterone to lower serum FP levels do not acquire amyloidosis as early in life as do normal hamster females. Also, down regulation of FP synthesis in female hamsters was associated with enhanced longevity, as female hamsters typically die of amyloidosis. Estrogen administered to male hamsters enhances expression of amyloidosis; however, only those estrogens which increase FP synthesis (such as diethylstilbestrol) will have this effect. Also, this amyloid enhancing effect of diethylstilbestrol can be inhibited by concomitant testosterone injections, a regime which inhibits FP synthesis. The sex hormone control of FP synthesis in the Syrian hamster provides a unique opportunity to examine the role of this pentraxin in amyloidosis and to show that in this one model the P component homolog is of primary importance in the deposition of amyloid. FP synthesis is under different control mechanism in other hamsters, for example, in Armenian hamster, FP is down regulated by estrogen administration; estrogen also induces an unusual acute hepatotoxicity in Armenian hamster and after 3 to 5 months, hepatocellular carcinomas are found in these estrogen treated animals. Induction of liver cancer by estrogen alone is unusual, and we are continuing our studies on the possible role of FP in this unique animal model of estrogen induced liver toxicity and cancer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00263-09 LPVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of the ADV Genome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. E. Bloom	Medical Officer	LPVD, NIAID
Other:	Dahn Clemens	IRTA	LPVD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.5	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

This is a non-clinical IIDEA project.

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to study various aspects of genome structure and function of the Aleutian mink disease parvovirus (ADV).

In the past year we have initiated studies on full-length chimeric clones of ADV. The strategy has been to replace segments of an infectious molecular clone of the nonpathogenic ADV-G strain with corresponding fragments of molecular clones from pathogenic ADVs derived directly from infected animal organs. Two of the 3 constructs assayed to date failed to produce infectious ADV after transfection and serial passage in cell culture; these were clones chimeric for either the 15-88 or the 43-65 map unit segments. A clone chimeric for the 65-73 map unit segment was able to replicate similar to the parent ADV-G. These results suggested that at least one determinant for permissive replication in cell culture maps to the 43-65 map unit portion of the genome, which contains the amino terminus of the virion proteins, the carboxy terminus of the non-structural proteins and sequences associated with one of the splices.

We have also begun to study the expression of ADV proteins in a vaccinia based eukaryotic expression system. A segment representing the spliced R3 mRNA that codes for both ADV structural proteins has been recombined into an infectious vaccinia virus. This recombinant vaccinia (VV-IL1) directed synthesis of both VP1 (p85) and VP2 (p75). The viral antigen was localized to the nucleus. Furthermore, lysates of VV-IL1 infected cells could be used as an ADV test antigen in counterimmunoelectrophoresis. Finally, parvovirus particles were observed by electron microscopy. Thus, all signals necessary for production and nuclear transport of both VP1/VP2 as well as those required for particle assembly were present.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00264-09 LPVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Structure and Function of Endogenous Retroviruses Expressed During Differentiation

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	J. L. Portis	Medical Officer	LPVD, NIAID
Other:	B. Chesebro	Chief	LPVD, NIAID
	M. Miyazawa	Visiting Scientist	LPVD, NIAID

COOPERATING UNITS (*if any*)

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00265-09 LPVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunobiology of Scrapie Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. E. Race	Research Veterinarian	LPVD, NIAID
Other:	B. Caughey	Staff Fellow	LPVD, NIAID
	K. Neary	Staff Fellow	LPVD, NIAID
	B. Chesebro	Chief	LPVD, NIAID

COOPERATING UNITS (if any)

Dr. A. Haase, Chief, Dept. Microbiology, University of Minnesota, Minneapolis, MN; Dr. Al Jenny, U.S.D.A., Ames, IA

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.5	.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

This is a non-clinical IIDEA project.

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Scrapie is a spongiform encephalopathy which under natural conditions affects sheep and goats. Similar diseases have been described in mink, mule deer, elk, and most recently, cattle. The disease in cattle has reached epidemic proportions within Great Britain's dairy herds. At least three human diseases, Creutzfeldt-Jakob disease, Kuru, and Gerstmann-Sträussler syndrome, are histologically indistinguishable from scrapie. Available evidence suggests that all of these diseases are caused by similar transmissible agents. The infectious agents which cause these diseases are particularly interesting because no nucleic acid genome has been associated with them. Interestingly, though, preparations that contain large amounts of scrapie infectivity do contain an aggregated proteinase K (PK)-resistant form of an endogenous protein, PrP. Experimental evidence suggests that PrP is important in disease pathogenesis. However, considerable controversy surrounds the exact relationship between PrP and the infectious agent of scrapie. A few investigators believe the PK-resistant form of the protein is itself the scrapie agent. Alternatively, it's possible that the protein could be a component of the agent or merely accumulate as a byproduct of disease.

We have utilized scrapie-infected cell cultures which we developed to study the scrapie agent and PrP. PrP exists in two forms, PrP-sen which is found in both normal and scrapie-infected animals and is easily destroyed by exposure to PK, and PrP-res which is found only in scrapie-affected animals and is partially resistant to destruction by PK. We have shown that a close association exists between the detection of PrP-res by immunoblotting and the presence of the scrapie agent. As a result we have continued to pursue the possibility that a quantitative assay for infectious agent can be developed based on detection of PrP-res in unknown tissue culture samples. Due to the recent outbreak of bovine spongiform encephalopathy and the potential risk that infected cattle might have for humans, we have initiated studies which could lead to a more rapid method for diagnosing spongiform encephalopathies. Other studies which are underway deal with the influence of the PrP gene on the incubation period and susceptibility to scrapie, mechanisms that might explain species tropism and studies seeking to determine factors which account for the conversion of PrP-sen to PrP-res.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00266-09 LPVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Structure of Murine Retroviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. H. Evans	Chemist	LPVD, NIAID
Other:	R. J. Monk	Senior Staff Fellow	LPVD, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

This is a non-clinical AIDS-related project.

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Retroviruses undergo frequent genetic alterations which in many instances may contribute to the pathogenicity of the viruses. Murine retroviruses, for example, readily undergo recombination with endogenous sequences of mice, leading to host range variants. Such variants have been directly implicated in the activation of cellular oncogenic genes. Variants which escape the host immune response or which exhibit an altered pathogenicity may be the result of point mutation of lentiviruses, such as the equine infectious anemia virus (EIAV) and the human immunodeficiency virus (HIV). This project is focussed on the occurrence and mechanisms of genetic alteration in retroviruses as well as the consequences of such alterations. In previous work we have utilized a number of monoclonal antibodies (mAbs) to identify and isolate recombinant host range variants from mice which were inoculated with murine leukemia viruses (MuLVs), or from mice which harbor endogenous MuLVs and spontaneously generate such variants. We have now identified three antigenic classes of the recombinant viruses which encompass all of the isolates examined. Structural analyses of the recombinants suggests that the three antigenic classes correspond to three distinct classes of endogenous gene sequences. Furthermore, we have found that different MuLVs recombine with different sets of endogenous sequences to preferentially generate distinct classes of viruses. Utilizing *in vitro*-constructed chimeric MuLVs, we found that the selectivity for a particular class of recombinant maps to the same regions of the genome previously found to influence the tissue-specific replication of the inoculated viruses. In preliminary experiments we have demonstrated pseudotyping of the inoculated virus resulting in an altered *in vitro* host range. Thus, it is possible that the generation of a particular recombinant influences the sites of replication of the inoculated virus. Alternatively, replication in different tissues may influence the class of recombinant viruses generated.

Another aspect of this project has focussed on the determination of the point mutation rate of retroviruses. Previous estimates of the point mutation rate of retroviruses have not been carefully controlled for the number of replication cycles and/or the target size for mutation. By strictly controlling the number of replication cycles and directly examining the virion RNA genome, we have determined that the mutation rate for a murine retrovirus is approximately 20-fold slower than previous estimates reported in the literature.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00418-07 LPVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunobiology of Equine Infectious Anemia Virus, a Retrovirus Model for AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. Maury	Staff Fellow	LPVD, NIAID
Other:	B. Chesebro	Chief	LPVD, NIAID

COOPERATING UNITS (if any)

Dr. Susan Carpenter, Department of Veterinary Microbiology, Iowa State University, Ames, IA 50011

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.8	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

This is a non-clinical AIDS-related project.

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The horse lentivirus, equine infectious anemia virus (EIAV), is a virus closely related to the human AIDS virus, HIV. We wish to use this virus as an animal model for HIV. However, to do this, more of the basic biology of EIAV needs to be understood.

Three projects are currently ongoing on the biology of EIAV. First is the characterization of infection and growth of EIAV in its target cell, the macrophage. In collaboration with Susan Carpenter, studies are underway characterizing regions within the LTR, the viral promoter, which are important for transcriptional regulation of the virus in macrophages. In concurrent studies, attempts are being made to identify proteins that are synthesized in macrophages that interact with LTR sequences.

Second, reconstruction of a molecular clone of EIAV is in progress. The molecular clone was derived by Dr. Carpenter while she was at Rocky Mountain Labs. This clone is not infectious, at least in part because of a stop codon in the envelope gene. Using site directed mutagenesis, we are replacing the stop codon and will determine if the molecular clone is now infectious. In parallel with this work, stop codons are separately being introduced into the three small open reading frames found in EIAV. These reading frames are believed to produce peptides that have similar functions to the small regulatory proteins found in HIV. Functional analysis of these mutations will follow.

In a third project, we are attempting to isolate a new field isolate of EIAV. The only two independently derived molecular clones currently characterized have been found to be quite similar at both the nucleotide and amino acid levels. To begin to understand the heterogeneity of EIAV, a third independent isolate will be analyzed. Knowledge of the sequence heterogeneity within the horse population at large has important implications for vaccine development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00468-05 LPVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biology of Human AIDS Retrovirus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Chesebro	Chief	LPVD, NIAID
Other:	W. Maury	Staff Fellow	LPVD, NIAID

COOPERATING UNITS *(if any)*

Julie Metcalf, NIAID, Bethesda, MD; Diane Griffin, Johns Hopkins University School of Medicine, Baltimore, MD.

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.6	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

This is a non-clinical AIDS-related project.

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has focussed on studying the ability of human immunodeficiency virus (HIV) to infect various human and mouse cell lines. The CD4 gene which encodes an important receptor for HIV infection was expressed in human and mouse cells and susceptibility of these cells to HIV infection was followed using a sensitive focal immunoassay (FIA) technique. The results indicated that HIV infection in certain human cells (HeLa) was related to the level of CD4 expression in different clones. However, in other human lines such as U87 astroglioma and SCL1 squamous cell carcinoma HIV infection was only rarely successful in spite of very high CD4 expression. Further studies indicated that HIV could bind to the CD4 on these cells, but HIV did not under go successful fusion and entry of the cells.

These results suggested that other cellular molecules in addition to CD4 were required for HIV entry of cells. This possibility was confirmed by showing that HIV could infect these human cells when the usual HIV entry mechanisms were bypassed by using viral DNA to tranfect the cells or by infecting with HIV genomes pseudotyped by packaging in envelope proteins of mouse retroviruses which use receptors other than CD4 for entry.

Similar experiments were also carried out using mouse cell lines expressing human CD4. In these mouse cells, there were at least two levels of resistance to HIV infection. The first was a block in entry similar to U87 and SCL1 human cells, but even when this block was bypassed by transfection or viral pseudotyping, mouse cells still had a markedly reduced level of HIV infection. Present data suggests that the reduced HIV expression in transfected mouse cells may be due to poor functioning of HIV promotor and enhancer sequences in these mouse cell cultures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00524-03 LPVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunologic Factors in Susceptibility and Resistance to Rabies Virus in Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L. L. Perry	Expert	LPVD, NIAID
Other:	D. Lodmell	Staff Scientist	LPVD, NIAID
	T. Ullrich	Staff Scientist	Ribi Laboratories, Hamilton, MT

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	0.7	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

This is a non-clinical IIDEA project.

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies on the immunologic mechanisms of susceptibility and resistance to street rabies virus (SRV) in mice are still being pursued using a model system developed by Dr. Donald Lodmell of LPVD. Previous investigations by my lab revealed that infection of susceptible mice is accompanied by severe involution of the immune system due to an adrenal stress response with excessive secretion of lymphotoxic corticosteroids. Further experiments revealed that starvation induced stress could not account for adrenal hyperactivity and that virus infection of the pituitary gland is a more likely explanation for adrenal dysfunction. Lymphoid involution appears to be a consequence rather than a cause of susceptibility to SRV in mice. These results have been published in the Joumal of Immunology (144:3552-3557, 1990).

The contribution of the peritoneal inflammatory response to rabies resistance was also investigated. Mice of susceptible strains (A strain mice) are known to have a number of defects in macrophage activation and chemotaxis that render them susceptible to a variety of bacterial infections. In our studies, peritoneal inflammatory responses were shown to be much greater in resistant as compared to susceptible strains of mice coupled with more rapid clearance of virus from the peritoneum. Induction of macrophage infiltrates in susceptible mice by pretreatment with trehalose dimycolate, the active component of an adjuvant under development for human use by Ribi Laboratories, induces protection in 60% of infected animals. Treatment is associated with an increase in peritoneal macrophages but not with increased anti-rabies antibody reactivity, suggesting that the early inflammatory response provides one level of protection against rabies lethality.

Finally, the mechanism of rabies resistance in SJL and BALB/c mice has been investigated by in vivo depletion of Th and Tc subsets using monoclonal antibodies. SJL mice apparently eliminate rabies virus in the periphery prior to penetration of the CNS, while BALB/c mice develop a CNS infection which they subsequently clear immunologically. Depletion of Th but not Tc cells reverses the resistance of SJL mice indicating a predominant role for antibody in virus elimination outside of the CNS. Depletion of Th in BALB/c mice also results in complete susceptibility to virus lethality. Interestingly, depletion of Tc in BALB/c but not SJL mice also results in ultimate susceptibility to rabies disease, apparently due to a failure to clear virus infection within the CNS. This is the first direct evidence for a role of Tc in elimination of rabies infection within the CNS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00550-02 LPVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

EAE: Immunoregulation of Relapsing Disease and Interaction with Rabies Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L. L. Perry	Expert	LPVD, NIAID
Other:	None		

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.35	0.15	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

This is a non-clinical IIDEA project.

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies in a murine model of EAE have not been vigorously pursued due to increased attention to experiments in the rabies system. Limited experiments revealed that reactivity to proteolipid protein, shown previously to develop spontaneously in mice immunized with myelin basic protein, is evident in spleen as well as in lymph node T cells and resides primarily within the spleen in late stages of disease. This may indicate that the spleen serves as a reservoir for memory cells in autoimmune mice. A manuscript to report these findings is currently in preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00551-02 LPVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular Interactions During HIV Infection *In Vitro*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L. L. Perry	Expert	LPVD, NIAID
Other:	None		

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.35	0.15	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

This is a non-clinical AIDS-related project.

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our studies in the HIV system have focused on the capacity of anti-DR antibodies to inhibit infection of normal cells in vitro. The absence of detectable effects on virus binding or syncytia formation led to an investigation of antibody effects on macrophages present within T cell cultures. First, methods were established for the removal of macrophages from peripheral blood lymphocytes using gelatin and plasma coated culture flasks. Next, it was shown that infection of T cells in cultures of PHA stimulated peripheral blood lymphocytes exhibited a dependence on the number of macrophages available within cultures, little or no infection occurring in the absence of added macrophages. Finally, the effect of anti-DR mAbs on primary infection of macrophage-depleted T cells was investigated in the presence versus the absence of added macrophages. Results revealed that anti-DR mAbs inhibited T cell infection only in the presence of added macrophages, indicating that the macrophage is probably the target of antibody activity. The virus strain used in these studies is strictly T cell tropic and does not appear to infect cultures of purified peripheral blood monocytes.The role of the macrophage in promoting T cell infection with HIV is not clear although it may be related to secretion of lymphokines that regulate T cell activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00580-01 LPVD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemistry of Scrapie Pathogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B. Caughey	Senior Staff Fellow	LPVD, NIAID
Other:	R. Race	Research Veterinarian	LPVD, NIAID
	K. Neary	Staff Fellow	LPVD,NIAID
	B. Chesebro	Chief	LPVD,NIAID

COOPERATING UNITS (if any)

Dr. R. Rubenstein, Virology Dept., Institute for Basic Research in Developmental Disabilities, Staten Island, NY; Drs. A. Dong and W. S. Caughey, Dept. of Biochemistry, Colorado State University, Ft. Collins, CO.

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Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.7	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

This is a non-clinical IIDEA project.

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

PrP is a normal endogenous protein of unknown function in brain and a variety of other mammalian tissues. In the brains of animals afflicted with scrapie and other transmissible degenerative neuropathies, PrP accumulates in a proteinase K (PK)-resistant form which can aggregate into fibrils and form amyloid-like plaques. There is substantial evidence that the formation of the PK-resistant PrP is scrapie specific and important in the pathogenesis of the disease. However, it is not yet clear whether the PK-resistant is the transmissible agent itself, a component of the agent, or a pathological byproduct of the disease.

Since the replication or pathogenesis of the scrapie agent may involve the production of PK-resistant PrP, we have continued studies of the properties and biosynthesis of both the PK-resistant and normal forms of PrP in tissue culture cells. We have determined that in scrapie-infected neuroblastoma cells, the two forms of PrP differ in their aggregation states, N-termini, turnover rates and sensitivities to protease and phospholipase treatments of intact cells. We have extended our studies of normal PrP biosynthesis to rat PC12 cells, and have found that the size of PrP precursors and products, the kinetics of PrP biosynthesis, and the linkage of PrP to the plasma membrane by phosphatidyl inositol are similar to those in mouse neuroblastoma cells.

Studies of the scrapie agent derived from mouse neuroblastoma cells have indicated that, like the brain-derived agent, it can be slowly neutralized by high concentrations of PK. This indicates that has an essential protein component. However the infectivity is resistant to treatment with a variety nucleases suggesting that it contains no nucleic acid or that the nucleic acid is protected by other components of the preparation.

It is possible that a conformational abnormality accounts for the characteristics of PK-resistant PrP. To investigate this possibility, we have purified PK-resistant PrP from scrapie brain tissue and are initiating secondary structure analysis of it by infrared spectroscopy. Ultimately, we hope to be able to ascertain whether its conformation differs from that of the normal PrP.

LABORATORY OF VECTORS AND PATHOGENS
Rocky Mountain Laboratories
Hamilton, Montana
1990 Annual Report
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ANNUAL REPORT
LABORATORY OF VECTORS AND PATHOGENS
HAMILTON, MONTANA
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
OCTOBER 1, 1989, TO SEPTEMBER 30, 1990

Members of the Laboratory of Vectors and Pathogens have continued their molecular dissection of important human pathogens during the past year. This multidisciplinary approach to microbial pathogenesis combines the fields of biochemistry, immunology, electron microscopy, medical entomology and molecular biology into a cohesive unit with strong interaction among its own members and with scientific units both inside and outside of the NIH system. This interaction together with an active seminar and guest researcher program insures that the best experimental approaches available are rapidly incorporated into the research programs of the laboratory. Since Lyme borreliosis is now the most common arthropod-borne disease in the United States, the Laboratory has made a major commitment of resources to the development of improved diagnostics and to a detailed understanding of how *Borrelia burgdorferi* produces its long list of both acute and chronic clinical features. The Laboratory of Vectors and Pathogens functions in three major experimental groups. Dr. Claude F. Garon serves as Laboratory Chief.

The Arthropod-borne Diseases Section, under the direction of Dr. Tom G. Schwan, has made important, new observations on the biology and molecular biology of *Borrelia burgdorferi* infection. In earlier studies, it was observed that continuous cultivation of a fresh uncloned isolate of the spirochete, in modified Kelly medium, caused a reduction in the number of detectible linear and circular plasmids and the loss of infectivity in the white-footed mouse, *Peromyscus leucopus*. Agarose gel electrophoresis and electron microscopic examination showed that a circular plasmid of about 8 kilobase pairs was lost at the same time that the strain became noninfectious, suggesting that genes encoding for factors responsible for infection may be present on this extrachromosomal element. Closer examination of supercoiled plasmids from ethidium bromide density gradients, revealed that a low passage of the uncloned strain Sh-2-82 of *B. burgdorferi* had at least six supercoiled plasmids ranging in size from 8.4 to 51 kilobase pairs. In addition, several of the supercoiled plasmids were observed by electron microscopy to form concatenated structures. Of the six supercoiled plasmids in strain Sh-2-82, four could still be detected after two years of continuous *in vitro* cultivation in modified Kelly medium. The two supercoiled plasmids of 8.4 and 8,8 kb that were lost after a maximum of 20 passages, shared a high degree of DNA sequence similarity, suggesting that their apparent instability may be a unique feature of these closely related plasmids. The Section next analyzed 14 unrelated isolates of *B. burgdorferi* from various geographical locations and tick or mammalian sources for their plasmid content and infectivity in Syrian hamsters, *Mesocricetus auratus*, and white-footed mice. All strains that contained either the 8.4 or 8.8 kilobase pair supercoiled plasmids were infectious, however, five additional infectious strains lacked both of these plasmids, indicating that these plasmids do not encode factors required for infectivity in our experimental system. Because the supercoiled plasmids are present in multiple copies, these plasmids were also examined for species-specific sequences that could serve as high copy number targets for a diagnostic DNA probe. Three *Eco*RI fragments that hybridized with multiple DNA fragments from *B. burgdorferi* were identified and cloned from a supercoiled plasmid-enriched fraction. The cloned sequences of 3.5, 4.2, and 4.3 kilobase pairs hybridized with most of the supercoiled plasmids in seven isolates of *B. burgdorferi*. None of the cloned sequences hybridized with chromosomal DNA from *B. burgdorferi* or with total DNA or supercoiled plasmids from *B. hermsii*, *B. turicatae*, *B. coriaceae*, *B. parkeri*, or *B. anserina*. These results indicate that the repeated sequences of DNA appear to be associated with plasmids and specific to *B. burgdorferi* (Schwan, Simpson).

Also participating in research on the molecular biology of the Lyme borreliosis spirochete is the Structural Pathobiology Section under the direction of Dr. Claude F. Garon. Careful structural analysis of the DNA content of several early passage and laboratory adapted strains of *Borrelia burgdorferi* has revealed, in addition to an approximately 1,000 kilobase (kb) genome, a unique collection of terminally cross-linked, linear and covalently closed, circular DNA molecules ranging in size from 1.5 to 50 kb in length. Often DNA contour length profiles appear to differ among isolates and to vary during laboratory passage. Since accurate structural characterization by agarose gel electrophoresis alone is not possible with an undefined

mixture of linear and circular molecules, nucleic acid electron microscopy techniques were used extensively in these studies to assess molecular form, sequence arrangement and genetic relatedness. Each of the linear molecules was shown to rapidly reanneal to linear duplexes after alkaline denaturation, and to form single-stranded, circular molecules measuring twice the length of the linears upon treatment with a combination of methyl mercury, glyoxal, urea and heat prior to mounting for electron microscopy. Both circle formation and rapid reannealing could be prevented by brief pretreatment with single-strand specific nucleases. Furthermore, after enzymatic removal of the terminal cross-link, separable single-stranded molecules could then be compared directly by conventional heteroduplex analysis techniques. Partial denaturation of either intact or single-strand specific nuclease treated molecules showed profiles suggesting that the highest G+C content in each of the linear molecules examined was located near the molecular termini. Similarly, contour length measurements of completely denatured linear molecules placed the cross-link at or very near the termini of the molecule. However, heteroduplex analysis of enzymatically nicked linears revealed no detectable inter-strand interaction. A technique was developed for the separation of terminal restriction fragments away from internal fragments using alkali denaturation, followed by a very brief incubation period to allow for concentration independent reannealing and final purification of snap-back, terminal fragments by ion exchange column chromatography. Heteroduplex analysis of purified terminal fragments also showed no evidence of inter-strand interaction suggesting little or no sequence homology among these regions of the linears. Examples of intra-strand interaction, however, were readily detected. One of the linear molecules, in addition to its covalently closed structure, appeared to possess a unique, inverted terminal redundancy of 2.3 kb located near the molecular termini of the 30 kb intact molecule. This homology, which appeared to be perfect or near perfect over the 2.3 kb duplex and present in each of the isolates examined to date, was demonstrable with purified terminal fragments as well. In addition, two prominent stemloop structures, structurally related to transposable elements in other organisms, were detected on two separate, linear DNA molecules isolated from *B. burgdorferi*. Inverted repeat sequences formed stems measuring 0.5 and 2.0 kb with intervening loops of 2.4 and 3.0 kb respectively. Smaller hairpin repeats (0.1-0.5 kb) also were visible along the length of linear DNA molecules. The significance of these structural features, if any, in *B. burgdorferi* has not been determined. All of the DNA molecules, with the exception of the 1,000 kb molecule, appear to be packaged in nuclease resistant form within, and are readily isolatable from, the membrane vesicles elaborated from the surface of *B. burgdorferi* cells (Garon, Dorward).

The Microbial Pathogenesis Unit, under the direction of Dr. Witold Cieplak, has focused on defining the structure-function relationships of two bacterial ADP-ribosylating toxins, pertussis toxin and *Escherichia coli* heat-labile toxin. These were studied using site-directed mutagenesis and photo-affinity labeling techniques. The studies during the past year have resulted in a detailed analysis of a number of mutants of pertussis toxin S1 subunit and a description of the functional aspects of various parts of the molecule. In prior studies, it was suggested that an arginine at position 9 of the S1 molecule might be important to the enzymatic activity of the toxin. There appears to be analogous arginine residues in areas of sequence similarity between the cholera toxin and *E. coli* heat-labile toxin. It was found that substitution of Arginine 9 of the S1 subunit with any of a variety of amino acids resulted in considerable decreases in ADP-ribosyltransferase activity. Further, photolabeling studies of mutant S1 analogs with radioactive NAD indicated that mutations at and in the vicinity of position 9 severely reduced the ability of the molecule to bind NAD and suggested an essential role in NAD binding. Mutation of this amino acid, along with a substitution at position 129 of the molecule, has recently been employed by another laboratory to create a mutant holotoxoid form of pertussis toxin that appears to be an excellent acellular vaccine candidate. In addition, studies have been designed to test the prediction that cysteine 41 of the S1 subunit is an active site residue of pertussis toxin S1 subunit. From the analysis of a number of mutant variants, it was concluded that while cysteine 41 is located in proximity of the NAD binding site, it has no essential role in catalysis *per se* and thus cannot be considered to be an active site residue. It was also determined that the carboxyl-terminus of the S1 subunit is likely involved in the binding of the acceptor G protein substrate during the enzymatic reaction. This feature was determined by the examination of carboxyl-terminal deletion mutants. The biological properties of some of the mutant holotoxins that have been constructed are in the process of being determined. Elimination of the ADP-ribosyltransferase activity by alteration of certain key amino acids, namely arginine 9 and glutamic acid 129, results in almost complete abrogation

of the ability of the toxin to potentiate the IgE response of mice, as judged by direct measurement of IgE levels to a co-administered antigen or by passive cutaneous anaphylaxis assays. Nevertheless, the mutant analog still retains the ability to protect mice from an intracerebral challenge from live *B. pertussis* organisms. This observation casts significant doubt on the role of the pertussis toxin-induced IgE response in the intracerebral challenge assay, and suggests that some other property of the toxin is responsible for allowing protection in this setting. Finally, as part of an effort to examine the potential role of invasion in the *Campylobacter* disease pathogenesis, the interaction of a pathogenic strain of *C. jejuni* with a human intestinal epithelial cell line has been examined. These analyses revealed that, while *C. jejuni* could be found bound to and within cells, the morphologic picture was one of passive uptake of the bacteria and not active invasion as exhibited by such other Gram negative enteric pathogens as *Salmonella* and *Shigella*. Moreover, the intracellular bacteria appeared, in many instances, to represent degenerate forms that were likely non-viable. Differential metabolic labeling studies have recently been initiated to determine if such intracellular forms are active. These studies will also address the question of whether *Campylobacter* spp. respond to intracellular conditions with the production of stress-response proteins. Additionally, it has been determined that *C. jejuni* does respond to heat-stress *in vitro* with the increased production of a number of proteins, several of which appear to be common to a variety of bacterial species (e.g. GroEL). These products have been identified using cross-reactive antisera (Cieplak, Cluff).

The focus of the Laboratory of Vectors and Pathogens has remained during the past year on the use of modern methods of molecular biology to define, in molecular terms, critical aspects of the host-pathogen relationship.

ADMINISTRATIVE

Several organization changes within the laboratory were proposed and approved during the past year. This included a name change from the Laboratory of Pathobiology (LPB) to the Laboratory of Vectors and Pathogens (LVP). The change was initiated to recognize the unique nature of the research being conducted and to accurately reflect the laboratory's research program

Drs. Willy Burgdorfer and John Munoz worked as scientist emeritus members of the laboratory during the year providing valuable service and support.

Guest Researchers in the Laboratory of Vectors and Pathogens have included Dr. Stanley Falkow (Stanford University School of Medicine), Dr. Lucy Tompkins (Stanford University), Dr. Kostis Georgilis (Tufts University, and Dr. Mark Klempner (Tufts University School of Medicine).

Visitors/Collaborators who spent varying amounts of time in the laboratory during the past year included:

NAME	AFFILIATION
Dr. Michael Felz	University of Georgia
Dr. Paul Duray	Fox-Chase Cancer Center
Dr. Pierre Lalouette	Salk Institute
Dr. Edward Huguenel	Molecular Diagnostics, Inc.
Dr. Howard Plummer	Bowling Green State University
Mrs. Karen Forschner	Lyme Borreliosis Foundation, Inc.
Dr. Alice Erwin	University of Texas Medical Center
Dr. Michael Koomey	University of Michigan
Dr. Denee Thomas	The Bowman Gray School of Medicine
Dr. Patrick Cleary	University of Minnesota
Dr. Russell Johnson	University of Minnesota
Dr. Yuri Chernukha	Gamaleya Institute, Moscow, Russia
Dr. Victor Kryuchechnikov	Gamaleya Institute, Moscow, Russia
Dr. Edward Korenberg	Gamaleya Institute, Moscow, Russia
Dr. Leonard Mayer	Centers for Disease Control
Dr. John Tagg	University of Otago, Dunedon, New Zealand
Dr. John Halperin	SUNY at Stonybrook
Dr. Camille Locht	Pasteur Institute, France
Dr. Tom North	University of Montana
Dr. William Granath	University of Montana
Dr. Rex Spendlove	Hyclone Laboratories, Inc.
Dr. Richard Feldman	DCRT, NIH, Bethesda

Joining the laboratory as IRTA Fellows during the year were Dr. Ken Gage (University of Texas), Dr. Rich Marconi (Roche Institute), Dr. Michael Konkel (University of Arizona), and Dr. Scott Samuels (University of Arizona). Completing IRTA Fellowships were Dr. Chris Cluff and Dr. Rex Thomas. Completing Visiting Fellowships during the year were Dr. Miroslaw Szulczynski and Dr. Yves Lobet.

The laboratory hosted the 45th International Northwestern Conference on Diseases in Nature Communicable to Man in August.

HONORS AND AWARDS

Dr. Claude F. Garon

Served on State Board of Directors, Center of Excellence in Biotechnology, Montana Science and Technology Alliance.
Faculty Affiliate - Division of Biological Sciences. University of Montana
Internal Advisory Committee. University of Montana Electron Microscopy Facility.

Invited speaker:

IV International Conference on Lyme Borreliosis, Stockholm, Sweden
Session Moderator, American Society for Microbiology, Anaheim, California
Department of Microbiology, Louisiana State University
Louisiana Society for Electron Microscopy 30th Anniversary Meeting, New Orleans, LA
Biomedical/Biotechnology Symposium, The Mansfield Center for Pacific Affairs, Helena, MT
Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY
Montana State Teachers Association Convention, Great Falls, MT
Hamilton Lions Club, Hamilton, MT

Reviewed manuscripts for:

Infection and Immunity

Dr. Tom G. Schwan

Invited speaker at Ball Memorial Hospital, Muncie, Indiana, October 11, 1989.
Invited speaker at Lyme Borreliosis Foundation meeting, Oakland, California, October 14, 1989.
Invited speaker at Nassau-Suffolk Hospital Council meeting, Smithtown, New York, October 23, 1989.
Invited speaker at Smith Kline/RIT, Brussels, Belgium, October 26, 1989.
Invited speaker at Southern California Branch meeting of American Society for Microbiology, San Diego, California, November 4, 1989.
Invited speaker and Symposium Chairman at Society of Vector Ecologists meeting, Norman, Oklahoma, November 14, 1989.
Invited speaker at 12th Annual Conference of Montana Dermatologists, Big Sky, Montana, February 24, 1990.
Invited speaker at U. S. Forest Service, Stevensville, Montana, March 19, 1990.
Invited speaker at Lyme Borreliosis Foundation meeting, Orlando, Florida, March 29, 1990.
Invited speaker at U.S. Forest Service, Eureka, Montana, April 4, 1990.
Invited speaker at IV International Conference on Lyme Borreliosis, Stockholm, Sweden, June 18-21, 1990.
Symposium convener and speaker at International Northwestern Conference on Diseases in Nature Communicable to Man (INCDNCM), Hamilton, Montana, August 13, 1990.

Committees:

Grant reviewer for Special Review Committee 90-06 for RFA 89-AI-14 for Lyme Pathogenesis, November 1989.
Ad hoc reviewer for NIAID TMP Study Section, April 1990.

Reviewed manuscripts for:

Journal of Medical Entomology
Journal of Wildlife Diseases

Invited to review for American Journal of Tropical Medicine and Hygiene

Dr. Warren J. Simpson

Invited speaker at 12th Annual Meeting of Society of Vector Ecologists, Norman, Oklahoma, November 14, 1989.
Invited speaker at Georgia Medical College, Augusta, Georgia, March 22, 1990.
Invited speaker at Lyme Borreliosis Foundation meeting, Orlando, Florida, March 29, 1990.
Presented at annual meeting of American Society of Tropical Medicine and Hygiene, Honolulu, Hawaii, December 1989.
Presented at annual meeting of American Society for Microbiology, Anaheim, California, May 1990.
Ad hoc reviewer for NIAID TMP Study Section, June 6-7, 1990.

Dr. Witold Cieplak

Meeting attended:

American Society for Microbiology, Anaheim, CA, May 1990
Gordon Research Conference on Microbial Pathogenesis and Toxins, Plymouth, NH, July 1990

Reviewed manuscripts for:

Infection and Immunity

Dr. Willy Burgdorfer

Recipient of the 1989 Bristol Award by the American Infectious Diseases Society, Houston, TX, September 16, 1989.
Invited to present lecture at Lyme Borreliosis Foundation, Inc. sponsored workshop "Borreliosis West '89," Oakland, CA, October 23-14, 1989.
Invited to present lecture at Nassau-Suffolk Hospital Council sponsored Lyme Disease Symposium, Smithtown, New York, October 23, 1989.
Invited to present lecture on Lyme disease at Glaxo Pharmaceutical sponsored symposium, Saint Vincent Hospital, Billings, MT, November 8, 1989.
Invited to present "1989 L. Vernon Scott Lecture" at University of Oklahoma Health Sciences Center, Oklahoma City, OK, November 13, 1989.
Invited as guest speaker at the annual meeting of the Society of Vector Ecology (SOVE), Norman, OK, November 12-15, 1989.
Invited to present lecture on "Tick-borne diseases of the Northwestern U.S.," Emergency and Medical Technician Association, Missoula, MT, November 28, 1989.
Invited to present telelecture on "Lyme disease: the present and future challenges," 1990 series, University of North Dakota Medical School, Grand Forks, ND, March 13, 1990.
Co-Chairman of the Borreliosis Foundation, Inc. sponsored symposium "Clinical Management and New Discoveries," Orlando, FL, March 29-30, 1990.
Invited to present lecture on Lyme borreliosis at Jersey Shore Medical Center sponsored symposium, "The Diagnostics and Treatment of Lyme Disease: 1990," Asbury Park, NJ, April 3, 1990.
Invited to present lecture on Lyme disease, Departments of Parasitology and Veterinary Research, Montana State University, Bozeman, MT, April 17, 1990.
Presented telelecture on Lyme disease, State of Idaho Department of Health and Welfare, Boise, ID, April 24, 1990.
Invited to speak on Lyme disease at University of Medicine and Dentistry of New Jersey sponsored Lyme Disease Symposium, New Brunswick, NJ, April 26-27, 1990.
Participated as instructor in Swiss Society of Microbiology sponsored workshop on rickettsial diseases, Sion, Switzerland, May 1-2, 1990.

Invited to present lecture on Lyme disease at Swiss Society of Microbiology Congress, Sion, Switzerland, May 3-5, 1990.

Invited to present lecture on Lyme disease at Veterinary Medical School, Bern, Switzerland, May 7, 1990.

Presented guest lecture on Lyme disease at Hopital de la Conception, Marseille, France, May 9, 1990.

Invited to present guest lecture at annual meeting of Georgia Rheumatism Society, May 18-19, 1990.

Invited to present lecture at the National Medical Library Association annual conference, Detroit, MI, May 23, 1990.

Awarded Honorary Doctor Degree of Science by Montana State University, Bozeman, MT, June 9, 1990.

Invited to chair workshop and to present paper at IV International Conference on Lyme Borreliosis, Stockholm, Sweden, June 18-21, 1990.

Served as instructor at 40th Acarology Summer Program, Ohio State University, Columbus, OH, July 1-6, 1990.

Invited to present lecture "Lyme disease - an update," Ohio Department of Health and Columbus Health Department, Columbus, OH, July 5, 1990.

Served as Secretary/Treasurer and Chairman of Organizational Committee for the 45th International Northwest Conference on Diseases in Nature Communicable to Man (INCDNCM), Hamilton, MT, August 12-15, 1990.

Invited to present lecture on tick-borne diseases (Rocky Mountain spotted fever, Lyme disease, Colorado tick fever, tick paralysis, ehrlichioses) to British Columbia Society of Medical Technicians (Canada) at convention in Kamloops, British Columbia, September 20-22, 1990.

Dr. John J. Munoz

Trustee of Stella Duncan Memorial Fund, University of Montana.

Selected to be listed in Who's Who among Hispanic Americans.

Participated as a consultant on pertussis vaccine.

Given Emeritus status by American Association of Immunologists, American Society for Microbiology, Reticuloendothelial Society, and the International Endotoxin Society.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00480-05 LVP

PERIOD COVERED
October 1, 1989, to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathogen-arthropod Interactions of Vector-borne Diseases Affecting Public Health

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Tom G. Schwan	Sr. Staff Fellow	LVP, NIAID
OTHERS:	W. J. Simpson	Visiting Associate	LVP, NIAID
	R. E. Thomas	IRTA Fellow	LVP, NIAID
	K. L. Gage	IRTA Fellow	LVP, NIAID
	R. H. Karstens	Bio. Lab. Tech. (Micro)	LVP, NIAID

COOPERATING UNITS *(If any)*
Center for Disease Control, Fort Collins, CO (A. M. Barnes, T. Quan); Stanford University, CA (K. McDonough and S. Falkow).

LAB/BRANCH
Laboratory of Vectors and Pathogens

SECTION
Arthropod-borne Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.7	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to develop molecular approaches to investigate pathogen-arthropod interactions of vector-borne diseases of human importance in the United States. The first objective was to develop a sensitive DNA hybridization probe to specifically identify *Yersinia pestis*, the causative agent of bubonic plague, in experimentally infected fleas. This was achieved in collaboration with K. McDonough and S. Falkow at Stanford University. Primers for use in a polymerase chain reaction (PCR) have been constructed and will be used to detect *Y. pestis* in experimentally infected fleas. The gene for Fraction 1 has been cloned in *Escherichia coli* using the λZAPII expression vector. The recombinant *E. coli* expresses significant amounts of the F1 antigen and protects mice that are challenged with virulent *Y. pestis*. We will evaluate the feasibility of this in developing a subunit vaccine for plague based on the F1 antigen. The possible role of soft ticks (Argasidae) in maintaining the plague bacillus in nature was examined experimentally using five species of the genus *Ornithodoros*. Most notably, 80% of *Ornithodoros hermsi* were infected with *Yersinia pestis* one month after feeding on infected mice, and viable *Y. pestis* were detected in 2% of the ticks after one year. Currently, we are completing our work on plague and will expand this project to examine tick-spirochete interactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00488-04 LVP

PERIOD COVERED
October 1, 1989, to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Ultrastructural Analysis of Antigenic Determinants in Pathogens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Claude F. Garon	Chief	LVP, NIAID
OTHERS:	S. F. Hayes	Bio Lab Tech (Micro)	LVP, NIAID
	D. Corwin	Bio Lab Tech (Micro)	LVP, NIAID
	W. Burgdorfer	Scientist Emeritus	LVP, NIAID
	D. W. Dorward	Staff Fellow	LVP, NIAID
	L. L. Lubke	Biologist	LVP, NIAID
	T. G. Schwan	Sr. Staff Fellow	LVP, NIAID

COOPERATING UNITS *(if any)*
LPVD, RML, NIAID; LMSF, RML, NIAID; LICP, RML, NIAID
Dept. of Medical Microbiology - Stanford University School of Medicine

LAB/BRANCH
Laboratory of Vectors and Pathogens

SECTION
Structural Pathobiology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.6	1.2	2.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project serves to consolidate and to expand our efforts at identifying, localizing, and characterizing genes and gene products which are important in host pathogen relationships. While we have used colloidal gold and ferritin labeling to identify important cellular components which are located on the surface of various pathogens, new techniques of cryofixation, cryoultramicrotomy and immuno-labeling will allow precise localization of important antigens which may be located inside the cell. A broad range of molecular techniques will allow tracking of antigens through synthesis, processing and genetic control modulation. Recently, intimate associations between nucleic acids and elaborated membrane vesicles have been demonstrated in a number of pathogenic microorganisms, including *Borrelia burgdorferi*. Efforts are underway to determine the structure, composition and possible role of these membrane vesicles in the pathobiology of *B. burgdorferi*. Initial experiments have suggested that membrane vesicles are composed of a unique subset of glycosylated proteins, which are detectable in urine, blood and tissues of infected animals. An electron microscope antigen capture/detection method has been developed and patented.

PHS 6040 (Rev 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00492-04 LVP

PERIOD COVERED

October 1, 1989, to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Basis for Infection by *Borrelia burgdorferi*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Tom G. Schwan	Sr. Staff Fellow	LVP, NIAID
OTHERS:	W. J. Simpson	Visiting Associate	LVP, NIAID
	K. L. Gage	IRTA Fellow	LVP, NIAID
	W. Burgdorfer	Scientist Emeritus	LVP, NIAID
	S. Czub	Visiting Fellow	LVP, NIAID
	M. E. Schrumpf	Bio. Lab. Tech. (Micro)	LVP, NIAID
	R. H. Karstens	Bio. Lab. Tech. (Micro)	LVP, NIAID

COOPERATING UNITS *(if any)* Southampton Hospital, Southampton, NY (A. MacDonald), CDC, Fort Collins (J. Piesman), Tufts University, Boston (K. Georgilis and M. Klempner), LMSF, RML (P. Rosa), Texas H. D., Austin (J. Rawlings)

LAB/BRANCH

Laboratory of Vectors and Pathogens

SECTION

Arthropod-borne Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.9	2.1	1.8

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☒ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to (1) determine if genes present on plasmid DNA of *Borrelia burgdorferi*, the causative agent of Lyme disease, control infectivity, (2) characterize these plasmids, (3) clone the infectivity genes and express those components that promote *Borrelia* infection in mammals, and (4) determine immunogenic properties of components responsible for infection, and (5) use recombinant DNA techniques to express *B. burgdorferi*-specific antigens to improve the serodiagnosis of Lyme disease. The reduction in the number of detectable plasmids with the loss of infectivity suggests that gene(s), encoding for components related to infectivity, may be present on one or more of these extrachromosomal elements. A 8.4 kilobase (kb) pair circular plasmid was first identified as a strong candidate for regulating infection. Numerous fresh strains of *B. burgdorferi* were examined for their infectivity and presence of the 8.4 kb plasmid, two strains that were infectious lacked this plasmid or sequences similar to it elsewhere in their genomes. Thirteen strains of *B. burgdorferi* were cloned by limiting dilution from the low passage Sh-2-82 and examined for their infectivity, plasmid profile, and reactivity with anti-*Osp*B monoclonal antibodies. One clone was noninfectious and had a unique and deficient plasmid profile compared to the other 12 clones. Investigations concerning *in vivo* antigenic changes in white-footed mice, the natural reservoir for *B. burgdorferi*, and white mice are continuing and have demonstrated that antigenic variation during infection is significant and may play an important role in the spirochete's ability to maintain persistent infections in spite of a pronounced humoral immune response by the host.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00552-02 LVP

PERIOD COVERED
October 1, 1989, to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunopathobiology of Bacterial Toxins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Witold Cieplak, Jr.	Sr. Staff Fellow	LVP, NIAID
OTHERS:	Christopher Cluff	IRTA Fellow	LVP, NIAID
	Susan G. Smith	Microbiologist	LVP, NIAID
	Marius G. Peacock	Microbiologist	LVP, NIAID
	Yves Lobet	Visiting Fellow	LVP, NIAID
	Ronald Messer	Biologist	LVP, NIAID

COOPERATING UNITS *(if any)* National Institute Environmental Health Sciences (Rodbell), Research Park Triangle, NC; SmithKline Biologicals (Locht), Rixensart, Belgium; Laboratory of Microbial Ecology, National Institute of Dental Research, NIH (Keith), Bethesda, MD.

LAB/BRANCH
Laboratory of Vectors and Pathogens

SECTION
Molecular Pathogenesis

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	1.2	1.6

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The structure-function relationships of two bacterial ADP-ribosylating toxins, pertussis toxin and *Escherichia coli* heat-labile toxin, were studied using site-directed mutagenesis and photo-affinity labeling. In the case of pertussis toxin, the information generated from the mutagenesis studies was used to create recombinant holotoxins for evaluation of immunobiological and toxic effects. The studies may lead to the development of suitable acellular vaccine constituents for prevention of pertussis (whooping cough), and for enteric bacterial diseases mediated by cholera-like enterotoxins.

Additional work involved studies of the relationship of the ADP-ribosyltransferase activity of pertussis toxin to another recently described cryptic activity of the toxin by using defined mutants. These studies are important to the evaluation of the suitability of the mutants as human vaccine candidates, and help clarify the structural bases for the various observed effects of pertussis toxin both *in vitro* and *in vivo*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00553-02 LVP

PERIOD COVERED
October 1, 1989, to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular Biology and Immunology of Pathogenic *Campylobacter* spp.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Witold Cieplak, Jr.	Sr. Staff Fellow	LVP, NIAID
OTHERS:	Christopher Cluff	IRTA Fellow	LVP, NIAID
	Susan G. Smith	Microbiologist	LVP, NIAID

COOPERATING UNITS (if any)
Stanford University Medical School, CA (Tompkins)
University of Arizona College of Veterinary Sciences (Joens)

LAB/BRANCH
Laboratory of Vectors and Pathogens

SECTION
Molecular Pathogenesis

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Campylobacter jejuni and *coli* have now been recognized as among the most frequent causes of bacterial enteritis in both developed and developing countries. Little is known about the manner by which these organisms cause disease, although there are data that support both toxic and invasion-mediated pathogenic mechanisms. Our work has focused on examination of the molecular properties of these organisms and their relationship to the virulence factors associated with disease production. Both the ultrastructural and physiologic properties of *Campylobacter* interaction with cultured human epithelial cell lines have been examined in efforts to assess the potential invasive properties of the organism. Oligonucleotide probes, specific antibodies, and enzymatic assays have been employed to identify potential cholera toxin-like molecules that might be involved in the production of diarrheal disease. Monoclonal antibodies to surface proteins have been produced and are being used to identify molecules that might be involved in adherence, colonization, and invasion. These antibodies are also being used to screen recombinant *Campylobacter* DNA libraries for the purpose of determining gene structure and investigating genetic regulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00554-02 LVP

PERIOD COVERED
October 1, 1989, to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structural Characterization of Microbial Genes and Nucleic Acid Molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Claude F. Garon	Chief	LVP, NIAID
OTHERS:	R. T. Marconi	IRTA Fellow	LVP, NIAID
	D. W. Dorward	Staff Fellow	LVP, NIAID
	T. G. Schwan	Sr. Staff Fellow	LVP, NIAID
	L. L. Lubke	Biologist	LVP, NIAID

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Vectors and Pathogens

SECTION
Structural Pathobiology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.3	0.6

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lyme borreliosis is now the most common arthropod-borne disease in the United States. *Borrelia burgdorferi*, the causative agent, has been isolated from humans, mammals, birds and arthropods and is cultivable in the laboratory. Careful structural analysis of the DNA content of several early passage isolates has revealed, in addition to a 1,000 kb linear genome, a unique mixture of terminally cross-linked linear and covalently-closed, circular DNA molecules ranging in size from 2 to 50 kilobases in length. Often DNA patterns appear to differ among isolates and to vary during laboratory passage. Given the number of molecules in this apparent extrachromosomal pool, the presence of genes for major surface proteins located on relatively small linear DNA molecules, the uniqueness of the DNA among isolates, and the structural features of these molecules, we have asked if this pool of molecules might not function as a collection of minichromosomes. This view has been supported recently by the description of a similarly segmented arrangement of DNA molecules in *B. duttonii* - an agent of relapsing fever. The objective of this project, therefore, is to define the genetic capacity of *Borrelia burgdorferi* in sufficient detail to begin the process of mapping genes and gene products that are important in the pathobiology of this microorganism.

ROCKY MOUNTAIN OPERATIONS BRANCH
Rocky Mountain Laboratories
Hamilton, Montana
1990 Annual Report
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Annual Report
Operations Branch
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1989 to September 30, 1990

Introduction

The branch provides all services necessary to the professional staff in the pursuit of their investigations. Fiscal support includes budget management, procurement, and initiating payments and follow-up on financial obligations related to purchases, contracts, staff and official guest travel, and expenses for conferences held at RML. Other support covers the following areas: personnel, communications, library services, secretary backup service, grounds care, custodial, security, media preparation, waste disposal including hazardous wastes and radioactive wastes, glassware cleaning, photography, animal rearing and care, motor pool, operation of the power plant, and full maintenance and minor laboratory renovations in every area except electronics.

The animal care and use program has been greatly upgraded this year. The Animal Care and Use Committee is now meeting monthly and taking an active part in reviewing and approving animal use protocols. A training program for personnel in the Animal Care Section has been initiated. Significant improvements have been made in caging for rabbits and hamsters. A microisolator system of rodent caging in the rearing unit (Bldg. 12) has been expanded and improved with the use of laminar flow work stations.

The physical plant has been improved with the completion of two contracts during the year. The controls in the boiler room have been upgraded to improve the efficiency of operating the boiler. The emergency power backup system has been greatly improved with the installation of two big diesel powered generators. The facility now has 100% electrical power backup in case of a power failure from Montana Power. We have also initiated a request to have the interior of the main complex repainted. We are in the process of upgrading overall security of the facility.

We have been involved with the hosting of three different meetings during the summer. In July the NIH Immunology, Virology and Pathology Study Section met in the seminar room and also conducted a workshop entitled "New Approaches to the Study of B Cell Growth and Differentiation". In August we hosted the annual meeting of The International Northwestern Conference on Diseases in Nature Communicable to Man. In September we hosted two field trips for the National Symposium on Health Research Career Opportunities for American Indians and Alaskan Natives entitled "Choosing the Paths to Tomorrow", held at the University of Montana.

We have continued to upgrade the communications system with the installation of FTS 2000 and expanding and adding enhancements to the LAN.

The Maintenance Section has performed a number of renovation projects for the Operations Branch, LMSF, LVP, LICP and LPVD in addition to their routine maintenance duties.

General Overview of the Responsibilities of Operations Branch

The fiscal and procurement department manages a budget of over 3.2 million. Payroll is not included in this figure. It covers only the purchase of services, supplies, and equipment used in the operation of the laboratories. Timekeeping and submission of the payroll are also handled in this unit.

Personnel initiates civil service and commissioned officer personnel actions, and advises on personnel matters. This department is also charged with operation of the Job Training Partnership Act in association with the local Montana State Employment Office when the program is active. Also handled by Personnel are persons under the following programs: Stay-in-School, Summer Aides, Student Volunteers, Special Volunteers, Visiting Program, and students studying for advanced degrees.

Most of the biological media used in the research laboratories is prepared in a special laboratory by a technician.

The Graphic Arts Department provides full professional services necessary in the laboratories with the exception of medical artistry.

The library provides a full range of services for the RML staff, i.e., selections and acquisitions, cataloging, circulation, inter-library loans, reference and bibliographic services, computerized data base searches, and preparing periodicals for binding.

The Animal Unit raises 2 strains of guinea pigs, 21 strains of mice, nine strains of hamsters, a colony of peromyscus and a colony of microtus. They are raising 84,800 animals annually for research from an average pool of 4,326 breeding stock (approximate figures). An additional 4,350 animals are purchased annually from outside sources, including mink, rabbits, rats, mice, hamsters and guinea pigs. After rearing, care is provided for these animals while they are under experiment. The most recent survey indicates an average daily holding of 16,152 small animals.

The Chief of the Branch is responsible for labor management work and administering the technical aspects of the contracts for Security, Custodial, operation of the Boiler Room and the Glassware Unit with the respective private contractors. Security is provided by a guard on duty every night and all day on weekends and holidays. Custodial services are provided in five laboratory buildings daily except weekends and holidays. Boiler Room operation provides heat, steam, compressed air, vacuum, and emergency power to the entire laboratory complex. Glassware is cleaned and sterilized in the Glassware Unit for reuse in the laboratories.

The Maintenance Department provides repair, service and renovation work in plumbing, electrical, sheet metal, carpentry, air conditioning, and refrigeration, including ultra-low temperature boxes. With the exception of electronic work, all maintenance is done by the staff. Also provided are demineralized and distilled water. A motor pool consisting of 10 vehicles is maintained. Grounds care including snow removal is provided.